Benefits of Arbuscular Mycorrhizal Fungi and Moringa to Lake Victoria (basin), Kenya; towards Sustainable Resource Management

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ABSTRACT

Strong foundations have not been laid regarding plant yield improvements in most agricultural ecosystems close to freshwater surfaces. Indeed mycorrhizal activities in regulating plant nutrient cycles belowground have been neglected. A perfect replacement to fertilizer has not been discovered suitable for such biomes. Benefits of arbuscular mycorrhizal fungi (AMF) mycobionts have been richly explored globally but practical techniques in implementation is challenging in the tropics. Being native to Lake Victoria ecology, Moringa species in symbiosis with AMF could build a model to help understand issues that can improve plant growth conditions and performance.

The aim of this study was to increase knowledge about the roles of microbial consortium which effect mycorrhization by using economically-targeted tropical Moringa plants. The study design covered biological soil fertility enhancement methods, fitting complex eco-sensitive large water biomes, since very little has been done in the region. The concept was to understand specific roles of AMF in plant nutrition, survival and yield enhancements in indigenous tropical plants viz *M. stenopetala* and *M. oleifera*, for forestation or alley-cropping in the environmentally degraded sites in Lake Victoria basin, Kenya. The specific purpose of this research was to evaluate plant-soil-mycorrhizal interactions under greenhouse experiments, by investigating nutrient availability signals in symbiotic and biomass factors used as indicators for improved nutrition in Moringa seedlings. The intention was to gain insight into microbial interactions in soil types sampled from representative regions (40 km transect limit) of Lake Victoria eco-zones.

The soils were divided into four blocks; three native soils and a standard substrate. The native soils were further divided into tillage frequencies such as high tillage (HT), in loamy oxisols from arableland, clay medium tillage (MT) alfisols sampled from sugar plantations farming regions and paddy low tillage (LT) types, of vertisols/histosols origin cored from a rice plantation area.

The first objective in this study was to perform a biotest on soil potential using *Plantago major* as trap-culture and indicator to harness autochthonous mycorrhiza and evaluate biomass in native soils under greenhouse experiments. This was designed to revitalize soil functions, determine plant performance through indicator plant and develop inoculum bank.

A higher germination of 62.5% in paddy LT compared to other native soils at 33% and 58% was realized. Mycorrhizal population corresponded with tillage intensity and $K^+$ abundance. The results from microscopic hyphopodium bioassays revealed high mycorrhizal colonization
identified from paddy LT soils. Distinct mycorrhizal structures mostly intra-radical vesicles, intracellular arbuscules, coils and hyphae were identified from the screened rootlets sampled from paddy LT. Higher germination rates and plant vigour was recorded from the paddy LT soils compared to other two native soils. A record of >90% degree of colonization was quantified on mycorrhized rootlets.

Developments from the first objective invited a follow-up investigation based on plant performance analyses. Questions to answer involved identifying factors responsible for biomass differences (e.g. soil properties or mycorrhization). Paddy LT soils, which were characterized by higher level of potassium (K⁺) mineral content, showed faster germination rates, growth and mycorrhization. For this reason extended K⁺ and AMF tests were performed on Moringa seedlings under controlled growth conditions. The results showed that AMF and K⁺ factors influenced tuber formation and growth of Moringa species.

In the next step, an experiment was performed to evaluate Moringa plant response to AMF and specific nitrogen fixing bacteria (NFB) in chickpea rhizobia (Rhizobium sp.) inoculation. Harnessed autochthonous and a cocktail of cultured AMF (Glomus intraradices, G. hoi and G. mosseae) inoculum was used on Moringa seedlings. Out of choice, native (paddy LT) and a standard soil type was set for analysing impacts of autochthonous and cultured AMF (referred to as “allochthonous” mycorrhiza in this study) on Moringa plants. AMF cocktail and harnessed mycorrhizal community, mostly of Glomus tenuis influenced Moringa plant growth concurrently at inoculum.

In attempts to investigate optimal growth promotion conditions, a fertilizer versus Moringa plant growth enhancer (MPGE) experiment was set. Being a plant growth promoter, MPGE and commercial fertilizer was concurrently added to the seedlings in limited proportions. MPGE is 100% Moringa plant material rich in plant nutrients and healthy to riverine and lake ecosystems. The use of Moringa as a multipurpose forest plant and growth enhancer in Agriculture, with high fertilizing ability makes Moringa a major candidate for sustainable resource management. For this reason, effects of commercial fertilizer and MPGE were tested. Plants established in MPGE showed improved growth in heights. Larger root tubers and comparatively larger basal stem diameters were observed on MPGE treated Moringa plants, although greater heights and faster growth was recorded on fertilized samples.

Finally, rhizotron methods were used in observing root developments. Morphometric fine root parameters such as root growth rates (RGR), specific root lengths (SRL) were analysed. Finer root formation with larger root surface in inoculated Moringa rootlets identified in Moringa rootlets, could assist in assessing improved nutrient uptake indicated by biomass returns.
In this study, soil factor and treatments promoted growth of *M. stenopetala* and *M. oleifera*. The results reveal that inoculum (AMF and NFB); K⁺ and MPGE are potential candidates in enhancing soil fertility in ecosystems associated with riverine or large water regimes. The findings are expected to contribute in optimizing production techniques related to low input agriculture.

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“Wise words are like deep waters”
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Dedicated to Daddy
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PLATES

Plate A1: (a) Loam HT + vermiculite (b) Loam HT (c) clay MT (d) paddy LT and inset standard/vermiculite at soil potential assessments; trap culture plant performance grown under ambient light, and regular irrigation in greenhouse multi-pot culture at native soil regeneration phase in 2009.

Plate A2: Photomicrographs (25x) of arbuscular mycorrhizal fungi colonization of rootlets at autochthonous inoculum development from native soils sampled from paddy LT. The data originates from greenhouse fine root cohorts.

Plate A3: Photomicrographs (25x) of arbuscular mycorrhizal structures identified in a range of host plants from native soils from Kenya. (a and b) tree-like intracellular arbuscules in ‘Arum-type’ infection unit >90% occupancy; (c) intracellular hyphal coils in ‘Paris-type’ (d) vesicles; (e) AM hyphae in contact with the root hairs (g) fungal attachment and appressorium.

Plate A4: Photomicrographs (25x) of AMF structures and dark septate endophytes (DSE) identified from the weeds and grasses. (a) Two entry points of AMF hyphae (arrows) and branched absorbing structure (asterix) of extraradical mycelia (b) Intraradical vesicles (arrows) of AMF, (c) and (d) DSE forming microsclerotia in graminoid root and root of weed from Kenyan soil seedbank.
ABBREVIATIONS

AMF: Arbuscular Mycorrhiza Fungi
DSE: Dark Septate Endophytes
HT: High tillage
LT: Low tillage
LUT: Land use type
MT: Medium tillage
MPGE: Moringa powder plant growth enhancer
NFB: Nitrogen fixing Bacteria in rhizobial-legume symbiosis
N: Nitrogen
P: Phosphorous
K⁺: Potassium
NH₄⁺: Ammonium
NO₃⁻: Nitrate
FRL: Fine root length refers to total length of root cohorts which are present during a defined period of time.
RTF: Root tip frequency is the number of root tips present along the length unit. This parameter can estimate the root growth rate in fine root distribution assessment.
RGR: Relative growth rate a measure for fine root production within a period of time.
R:S Root shoot ratio
SOM: Soil organic matter
SMC: Soil moisture content
SRL: Specific root length calculates total length of fine root cohorts associated with a weight unit of dry biomass which can also be applied in measuring diameter.
SRA: Specific root area is the surface area (soil root interface) of unsuberized roots present which guaranty acquisition to plant water and nutrient elements.
ST: Standard soils (vermiculite/peat)
TDR: Time-domain reflectometry
1. INTRODUCTION

1.1 Resource conservation

The need to conserve natural resources, reduce or reverse degradation and the surging costs of fertilizers (Siddiqui and Pichtel, 2008) encourages innovative, long term solutions possible in biological methods to improve and sustain soil fertility. Depending on pressure on land, mineral turnover may not accommodate increased plant mineral needs. Economically-targeted Moringa forest species, in symbiosis with arbuscular mycorrhizal fungi (AMF), can aid reforestation in degraded regions of Lake Victoria in a simpler way. Mycorrhiza is one of the successful low in,put technologies useful in enhancing plant mineral bioavailability. Intensive cultivation in Lake Victoria basin mine essential minerals from soil, leaving natural biogeochemical processes unable to compensate the huge phosphorus (P) deficit at erosion, leaching and harvests. Beneficial mycorrhiza enable these processes through dissolution of parent rock material in established soils (Bellgard and Williams, 2011), in absorption and delivery of nutrients from soil matrix to hosts with an obligatory return of carbon from plant-host to fungus (Dighton, 2003) in belowground trade. AMF, characterized by arbuscules are the most common types in tropical soils (Sieverding, 1996). The arbuscules are thought to be the centre of P mineral transfer, enabling a wider surface area for nutrient absorption (Schnepf et al., 2007).

However situations such as water stress, invasive species or ruderal weeds may alter benign association in mycorrhization. Environmental conditions fluctuate enough that any given association moves back and forth along mutualism-parasitism continuum (Bellgard and Williams, 2011), interfering with organism fitness, resiliency and functions. Currently dark septate fungal endophytes (DSE) are gaining attention in mutualistic symbiosis, parasitism or even antagonistic impacts in the mycorrhizosphere, identified frequently in grasses of most tropical plants (Cao et al., 2002; Akello et al., 2007). Jaison et al. (2012) recently reported that 33% of fruit crops in their study that was associated with DSE fungi similarly contained AMF, requiring further investigations based on endophytic roles in mycorrhization.

Studies show that mycorrhiza is likewise mutualistically associated with N$_2$-fixing prokaryotes encouraging dual inoculum in effective mycorrhizal management practices. AMF enable biochemical changes in plants by increasing various enzymatic activities applicable in phytoremediation, Mathur (2007). Acid phosphatase and nitrate reductase are important
enzymes of phosphorus and nitrogen metabolism (Panwar and Vyas, 2001) effecting N and P uptake in plants.

A major problem in Lake Victoria is eutrophication caused by soluble P and N either from commercial fertilizer in heavy agriculture or farmer available animal wastes; ammonia (NH$_4^+$), applied on croplands situated in the riverine or ground/surface water catchment areas. Madadi (2007) analysed total reactive P, hydrolysable P, sediment exchangeable P, sediment bioavailable P and soil available P from the Kenyan side of Lake Victoria. The soils from the catchment areas of the lake contained 10 to 100 times higher P concentration compared to sediments and water samples. Their data record shows P flux in the basin where atmospheric deposition and land runoff account for 90 % of P and 94 % of N inputs from agricultural fields. Lake Victoria basin with 30 million, an average population of 297,97 and 635 persons per km$^2$ respectively (Olago et al., 2009) faces persistent deforestation in favour of conventional agriculture, increasing resource degradation especially in soil and water regimes.

Agriculture in forestlands, being the main driver in landuse change in Lake Victoria basin (Olago et al., 2009), reduces vegetation with adverse effects. According to Mongabay (2000) tropical forest ecosystem databank, only 6.2% or about 3,522,000 hectares of total area 56,914 km$^2$ of Kenya is currently forested. Less than 2% is fully forested with humid canopies in Mau forest, an Af fromontane type and a single rainforest system in Kakamega, western Kenya. Of this, 20 % or roughly 704,000 hectares is classified as primary forest. 8.01% is used for food and other permanent crops 0.97%, pastures and herbaceous forage crops. Change in forest cover between 1990 and 2000, indicated that Kenya lost an average of 12,600 hectares of forest per year with an average annual deforestation rate of 0.34%. Between 2000 and 2005, the rate of forest change decreased by 1.4% to 0.34% per annum. In total, between 1990 and 2005, Kenya lost 5.0% of its forest cover, or around 186,000 hectares. In this, a loss of 38,000 hectares of its primary forest cover was lost. Measuring the total rate of habitat conversion (defined as change in forest area plus change in woodland area minus net plantation expansion) for the 1990-2005 intervals, Kenya lost 2 % of its forest and woodland habitat. Waiswa (2011) recently realized a steady decline of forest cover in the lake crescent from about 9.0% in 1989 to 4.4% in 2009, using unsupervised classification of satellite imagery to document Lake Victoria forest dynamics. Deforestation increases desertification and ecosystem degradation, exposing the soils to direct solar radiation and higher soil temperature which may alter soil biology and reduce microbial activities (Deyn et al., 2011). Likewise, sporulation and reproduction of mycorrhiza are directly affected by seasonal dynamics, confirmed by large seasonal fluctuations in number of spores of AM fungi
Sober reforestation plans invite sensitive methods in forestry practices at climate change signals around large watersurfaces. Large scale changes in the structure and function of tropical forest ecosystems, whether from pressures of development or impacts of drought can alter the balance in the annual exchange of carbon with far-reaching implications for the pace of climate change (Norby, 2011), through the missing sink. Forests promote soil microbial abundance due to their undisturbed habitats that facilitate biochemical nutrient flows compared to frequently-tilled cropland (Borie, 2006). Unfortunately P flow in tropical soils has not been well understood. Bühler et al. (2002) observed a radio-labelled P recovery time in organic P fractions test in a very short time (two weeks), in P mineralization process. However Rao et al. (2004) documented conflicting reports indicating that this element did not accumulate in organic phosphorous pool in time, making P element still unavailable to plants in such soils. The study supports Karanja et al. (1999) who observed that P application on *G. robusta* seedlings had negative responses, justifying biological method preferences in tropical soil management.

Devoid of scientific validity, some tropical soil myths describe the soils as weak in cations, incapable of retaining nutrients against leaching (Sanchez, 1999), confirmed by several records of P limitations in the region. Friesen et al. (1997) analysed chemical fractionation of soil organic P, indicating that applied P fertilizer moved preferentially into labile P pools, a rather slow input compared to organic input into the soils. Niang et al. (2002), Smestad et al. (2002) however argued that even in the absence of external inorganic P inputs, annual yields of crops (mycotrophic obligates e.g. maize–legumes), harvests were significantly higher in fallow rotations than in continuous cropping in these soils. Jakobsen et al. (2001) likewise documented on P transport efficiency in land augmentation techniques that encourage plant-microbial interactions for soil regeneration in tropical soils. Jama et al. (2000) in their studies in western Kenya also observed that nitrogen fertilization without simultaneous P addition often failed to increase yields.

Despite scepticism in forest establishments due to time and space required in the practice, Moringa is a fast-growing multipurpose species that can sustain environment in simplicity when established either at mixed-/or agroforestry levels. Optimal use of Moringa and beneficial mycorrhiza can be applied in restoration of degraded ecosystems. Although several plant species, owing to their rooting architecture, mycorrhizal presence and soil P from recalcitrant compounds (George et al. 2002) adjust to P shortage by exuding organic acids that stir adsorption to hydrolyze and release P (Smit et al. 2000; Tawaraya et al., 2006), the activity may not offset P element at loss.
1.2 The problem

Approximately 14% of Kenya is agriculturally viable. The rest are water and arid lands, mostly occupied by national reserves. With population growth higher than the rest of Africa, Lake Victoria basin occupancy has outpaced the continental average, reflecting growing dependency and pressure on the lake’s resources (FAO, 2008). Sustainable use of these resources is challenged by overpopulation calling for sound techniques in land and water resource conservation.

Case-in-point: Lake Victoria

Lake Victoria, East Africa, is the second largest lake in the world after Lake Superior and the largest freshwater biome in the world with an area cover of 68 000 km$^2$, a volume of about 2,750 km$^3$ of water. The lake occupies a shallow depression in the East African Plateau, with a maximum depth of 84 m and an average depth of 20 m. Kenya (6% or 4,100 km$^2$), Uganda (45% or 31,000 km$^2$) and Tanzania (49% or 33,700 km$^2$). It is situated between latitudes 0°30' N and 2°30' S and between longitudes 31°50' E 34°10' E with a surface altitude of 1,135 m above sea level. The shoreline is currently 3,440 km covering an area of 68,800 Km$^2$. Its trough is fed by direct precipitation (85%), with a mean rainfall of 1780 mm and inflow streams (15%) of its water mass. The riverines originate from Lake Victoria’s catchments in Kenya highlands with 184,000 Km$^2$ surface areas.

Regrettably L. Victoria is a major sink to leached, eroded materials and pollutants from agriculture and industries (Fig. 3). Over 90% of farming activities are based on rainfed agriculture (Koutsouris et al., 2010) with less erosion control. Heavy agriculture existing in Chemelil sugarbelt, Kisii highland and Ahero rice fields within the basin has direct impacts on the lake and its catchments. Agriculture causes soil infertility, degradation and heavy metal accumulation. Heavy metal detection studies in this region revealed that Pb and Cd in solutes, ranged from 6-10 times (Ongulu et al., 2009) higher than recommended 0.003 mg/L and 0.05 mg/L limits (WHO, 2004). Water quality in Lake Victoria continues to decline due to increased inflow of nutrients into the lake (Njiru et al., 2002). Algal biomass has increased 8-10-fold while chlorophyll-$a$ concentration varies between 2 and 13 mg m$^{-3}$ in the main lake and between 8.8 and 71 mg m$^{-3}$ in the inshore waters (Lung’ayia et al., 2000), compared to 1.2-5.5 mg m$^{-3}$ and 30 mg m$^{-3}$ in the same waters in 1960 and 1961, respectively (Talling, 1965). In the past soil infertility was overcome by bioaugmentation in fallow rotational cropping. However market needs and priorities changed favouring economically-targeted monocultures in continuous cropping systems. The land is now frequently-tilled and fallow.
rotational techniques no longer fit into this system. Tillage affects plant and soil microbial biodiversity important in maintaining soil health and fertility (Borie, 2006). Fertilizers are applied in soils to improve yields. However fertilizer chemicals especially N and P are highly soluble and easily enter hydro-systems endangering aqualife. Biological methods and selected mineral-rich plant materials such as MPGE (tab.1) which are not yet fully explored are sustainable ways of improving soil fertility in regions near freshwater biomes.

1.2.1 Research Concept: *P is central in resource management*

![Diagram of nutrient flow](image)

**Fig. 1** Concept of study, demonstrating nutrient flow from agricultural fields into lake system with a reverse flow possibility by alternating fertilizer use with biological methods in soil fertility improvements.

1.2.2 Rationale

Although Oberson et al. (2005) commended high P mineral sorbing characteristic in the tropical western Kenya soils, they observed a persistent P constraint situation. Hinsinger (2001) had described this earlier as a peculiar mineralogical and ambient geochemical mechanism of the soil structure, which favours strong P ion-retention onto the solid constituents contrary to soil solute state. The condition probably exacerbates high P loss into the water system, as the soils in croplands diminish in fertility and degrade. Further soil degradation is enhanced by high tillage and bare soils in the basin (Fig. 3), with physico-
biochemical impacts. Is it possible to achieve the plant recommended critical mineral status in the soils and at the same time optimally aid the recovery of the L. Victoria in a reverse system flow? (Fig.1). Sanchez et al. (1999) illustrated macronutrient application level (0.8 million tons/year) compared to a loss of up to 4.4 million tons/year, almost ten times the input in African agro-ecosystems. AMF activity enhancements and addition of high mineral content of MPGE in soils may help rescue the endangered ecosystem. In this project, a combination of symbiosis and MPGE application is perceived as a possible method of improving soil fertility in L. Victoria ecosystem.

1.2.3 Goals and objectives
The study aims at understanding roles played by mycorrhiza and Moringa as eco-sensitive soil upgrading techniques applicable in forestation/alley-cropping. The overall goal is to achieve optimal sustainable methods in improving soil fertility regimes, plant yields and livelihoods in a multidisciplinary approach in combined sustainable resource (agriculture, forestry, soil and water) managements.

The first objective of the study was to perform a biotest 1. to revitalize infertile and/or degraded soils 2. to evaluate performance through plant yield across representative Kenyan soil types 3. to harness native mycorrhiza.

Next objective was focused on assessing the reasons for plant performance differences in objective 1, in a follow-up experiment. Questions to answer were; 1. What factors are behind biomass differences? AMF or Potassium?

The third objective was based on effects of treatments on plant performance ranging from inoculum (AMF, NFB) to soils as well as comparing effects of allochthonous versus autochthonous mycorrhizal effects on Moringa plant yields.

The fourth step was focused on evaluating effects of MPGE and commercial fertilizer on plant yields.

Finally non-destructive observations of root developments were performed to assist in analysing nutrient uptake using rhizotron methods. This was to assess growth parameters such as root growth rates, specific root lengths, surface area, root volumes, root tip frequency etc.
CHAPTER TWO

2. LITERATURE REVIEW

*Plant Nutrients, Moringa and Mycorrhiza*

2.1 Plant nutrition

For optimal plant production, critical nutrient level in soil is necessary to achieve quality plant performance. MacNicol and Beckett (1985), Pilon-Smits et al. (2009), White and Brown (2010) defined this concept of “critical nutrient level”, as the concentration of the nutrients in a diagnostic tissue that allows up to 90% maximal yield, while toxicity is realized at > 10% decrease in plant performance. Increased production on less productive lands requires large amounts of mineral restocking to replenish loss. Agriculture extracts essential elements to plants such as N, P and K from the soils through accelerated erosion, leaching, and harvests. The macro-/micronutrients, about 17 elements including gases CO₂, O₂, and H₂O in higher plants (Marschner, 1995), are important in plant nutrition. The macronutrients (N, P, K, Ca, Mg, and S) are required in large amounts while micronutrients or trace elements (Fe, Zn, Mn, B, Cu, Mo, Ni, al, Na and Cl) are needed in small doses. Other elements are recurrent in soils but not so useful in plant nutrition, such as copper (Cu), chromium (Cr), lead (Pb), cadmium (Cd) and other minerals, most of which enhance heavy metal accumulation and contamination in soils. While “critical mineral content” level achievement is important; deficiency stunts growth and excess is toxic to plants and biodiversity.

Nitrogen occurs mostly in ammonium (NH₄⁺) and nitrate (NO₃⁻) forms. These are naturally in Isotopes N¹⁵ at 0.3663% and N¹⁴ at ≥99.6337% respectively in the soils. Below these levels, a depleted status is realized while above these percentages, N mineral enrichment is achieved. Nitrogen affects the absorption and distribution of practically all other elements and works in cohesion with other macronutrients effectively in promoting plant growth. N and P are part of structural, genetic and metabolic compounds in plant cells, that aid photosynthesis, enable biochemical reactions in energy transfer and other important functions to plants. P (P₂O₅) effects growth and stimulates development of floral and fruit buds. P deficiency is known to affect organogenesis and biomass partitioning in favour of the root system, affecting root:shoot ratio in plants (Poorter and Nagel, 2000). Phosphorus stress results in dwarfed plants, delayed maturity, and reduced yield (Snyder and Stewart, 2003) but in excess, toxic (Romera et al., 1992). K is the most abundant ion in higher plants, essential in enzyme
activation, protein synthesis, photosynthetic band-mediating osmoregulation, stomatal movements and tropisms, effecting deeper and larger root systems through cell enlargements in plants (Marschner, 1995; Silva, 2004). Apart from phloem solute transport and maintenance of cation:anion balance in the cytosol and vacuole (Chaves et al., 2005), K is similarly associated with plant nutrient translocation and concentration of other macronutrients in soil solution (Yanai et al., 1996; Ashley et al., 2005).

Naturally, biogeochemical processes should be able to replenish soils with sufficient minerals at loss. Unfortunately these processes are slow, unable to compensate the nutrient deficit at erosion, leaching and harvests (Liu, 2006). The result is a steady increase in commercial fertilizer use which threatens environment and health. N and P fertilizers in agriculture are contributors to eutrophication processes in surface and ground water in developed and developing nations (Conley et al., 2009). 63% of total N from anthropogenic sources is in radio-/photo and biological organic N in mineral N in NO$_3^-$ and NH$_4^+$ forms useful to plants, but may impact the environment negatively (Dobermann, 2005). Gases (NO$_x$, NH$_3$, and N$_2$O) of N element are chemically active in the troposphere with greenhouse effect (Galloway et al., 2008) that may cause acid rains, global warming and other irreversible environmental impacts. From anthropogenically applied nutrients, those “on the run” pollute systems. The desire to keep up with rapidly growing population needs may surpass ecosystem’s carrying capacity. Misjudgements on yield increment targets often occur in less-controlled systems, leading to excess fertilizer additions with gross health impacts.

2.2 Biogeochemical cycles

N, P and K dynamics, distribution and availability important in plant nutrition are ascribed to biogeochemical cycle. The process is known to release chelating compounds such as organic acids, siderophores and acidifying molecules by plants in mineralization processes. Siderophores or phytosiderophores are metabolic byproducts of fungus. These are low molecular chelators with high affinity to Fe$^{3+}$ that are able to make complexes with other metal ions such as Zn, Cu, Mn, Cd, Cr, Ni and Al (Buyer et al., 2002; Aliasgharzad, 2009) in soil nutrient enrichment and availability to plants, in element mobilization and mineralization (Fan et al., 2005) processes.
2.2.1 Nitrogen cycles

2.2.2 Nitrogen fixing bacteria

The mineralization of soil organic N through nitrate to gaseous nitrogen by soil microorganisms is a major component of global nitrogen cycling. Four main microbial processes in biogeochemical cycles responsible for N pool fluxes include nitrogen fixers, nitrifying and denitrifying bacteria. N-deficiency limits plant growth. Nitrate uptake in several higher plants is mediated by different transport systems. A high affinity saturable system operates at low NO$_3$ concentrations, whereas a low affinity linear system operates at high NO$_3$ concentrations (Cerezo et al., 1997). Nitrate reductase catalyzes the first step of nitrate assimilation in higher plants and algae, which appears to be a rate limiting process in the acquisition of N in most cases (Flores et al., 2002). Through a complex interaction with bacterium encodes, the basic enzymatic machinery for converting molecular N into ammonium, plus a number of genes required for the symbiosis is achieved (Heckman, 2006). Symbiosis can produce N yield of 100-300 kg N ha$^{-1}$ yr$^{-1}$ (Heckman, 2006) while the rest come from soil reserves (Johnston and Steen, 2000). Microbials such as mycorrhizal fungi that colonize plant rootlets release inorganic nutrients from minerals such as apatite or biotite and transfer the nutrients to their hosts in symbiosis. These are responsible for effecting plant nutrients (N and P) in tripartite synergistic symbiosis.

2.2.3 Phosphorous cycles

Generally, P occurs in three cycles; in land/soils, inland water surfaces/ riverines and in ocean sediments (Fig. 2). Through biogeochemical processes P is released into the soils. Total amount of annual P losses from the lithosphere into freshwaters is estimated at 18.7–31.4 MMT P/yr (Liu, 2006). It is known that human intensified landuse causes a degree of P flux in the environment. Globally, out of 10.5 MMT of P applied into the soils each year, nearly one half is lost (Smil, 2000; Compton et al., 2000). A huge amount of p in the terrestrial soils approximated at 90×10$^3$–200×10$^3$ MMT of P is extracted from the soils (Emsley, 1980; Richey, 1983; Filippelli, 2002) totalling to 13 MMT P/yr of P loss each year. Organic P cycling between soil, plants, animals and microbials range from 30% to 65% of total P, and accounts for up to 90% in tropical soils (Condron and Tiessen, 2005). This activity is enhanced mostly by P mediating microbials that play important roles in P turnover. Microbials and other enzymic activities important in weathering processes substantially release organic P in due time unlike weathering mechanisms, enabling timely soil P adjustments into the global system (Liu, 2006) of P flow. Effects of delayed or low P imply
poor plant performance and low yields. In many ways P element limitation in soils cause anthropogenically induced excess additions in the soils depending on land sizes and plant species. A small proportion, perhaps 15–20% of the total amount of P in the plant, comes directly from the fertilizer applied to the crop (Liu, 2006).

Fig. 2 The human intensified global phosphorus cycles; Sources: Liu et al. (2004), Richey (1983), Smil (2000).

2.2.4 Potassium availability and nutrient dynamics in soils
Soil potassium (K) is ascribed in four pools (Ashley et al., 2006). 90 to 98% are in form of feldspar and mica unavailable to plants. The first is non-exchangeable (fixed K\(^+\)) from 1 to 10%, associated with the ratio 2:1 of clay minerals. Secondly, exchangeable K\(^+\), the third 1 to 2% of available potassium is based on cation exchange sites or in solute form. The fourth is found in organic matter and within soil microbial population; however this is minor in accomplishing plant K\(^+\) needs. Water-soluble soil concentrations of K\(^+\) range from 0.04 to 3%, the release of exchangeable K\(^+\) is often slower than the rate of K\(^+\) acquisition by plants (Sparks and Huang, 1985) and consequently, K\(^+\) content in some soils is often very low (Pretty and Stangel, 1985; Johnston, 2005). Plant K\(^+\) status may further deteriorate in the presence of high levels of other monovalent cations such as Na\(^+\) and NH\(_4\)\(^+\) that interfere with K\(^+\) uptake (Spalding et al., 1999; Qi and Spalding, 2004). K\(^+\) uptake is optimal on warm,
moist soils that are well aerated and have a slightly acidic to neutral pH aided by the presence on mycorrhizal communities (Sala et al., 2000). Soil moisture content variations explain K⁺ fluxes depending on soil heterogeneity. In dry soils, bulk K⁺ content is normally higher with restricted mass flow and diffusion (Seiffert et al., 1995; Liebersbach et al., 2004; Samal, 2007), compared to solute states.

2.2.5 Effects of fertilizer on the environment

Despite the big size of arable land, up to 10% of fertilizer consumption amounting to 1.3-million tons is consumed in sub-Saharan Africa, a little over 1% of absolute levels and doses which is still low in the global scales (Naseem and Kelly, 1999) comparatively. This implies that fertilizer need not be a problem in sub-saharan Africa but congestion around water regimes increases agrochemical wastes with health problems. In some areas plant minerals, from recalcitrant compounds and conventional fertilizers tend to raise levels beyond plant requirements, seeping into water regimes. Some of these minerals (Cd, Zn, Pd, Ni and Cr) accumulate as heavy metal stockpiles in agricultural soils, infiltrating water surfaces with risks of resource contamination. Cadmium phosphate ore is mined mostly in the tropics. Cd is one of the most toxic heavy metals without any known biological function in soils (Sajidu et al., 2006). Heavy metal accumulation can modify essential protein structure or replace an essential element in plants causing chlorosis, growth impairment, browning of roots and inactivation of photosystems among others (Görhe and Paszkowski, 2006). Unfortunately heavy metals end up in most tropical soils without viable degradation budgets. Decadmating such ores are costly and heavy metal degrading technology is still speculative. Managements of such metals involve taking other alternative fertilizers and avoiding double expenses in imports of the ores and decadmiation techniques. Attempts to reduce heavy metal management expenses by Germany as an example, is the drastic reduction of phosphate fertilizer use. Considering 70 kg P₂O₅ ha⁻¹ a⁻¹ of fertilizer application in 1980 (Sauerbeck, 1985; IVA, 2003), a reduction to the current 19-20 kg P₂O₅ ha⁻¹ a⁻¹ is a significant step in dealing with impacts of heavy metal presence in soils.

Apart from heavy metal presence in the soils and water regimes, agriculture cause persistent organic pollutants phenomena which are expensive to extract, filter and stabilize or biodegrade in affected soils or water surfaces. Several conventions have been set to monitor and control chemical accumulation in ecosystems. Stockholm Convention and United Nations Environment Programme (UNEP) on persistent organic pollutants adopted in 2001 and amended in 2004 lists “dirty 12” substances majority belonging to the phenyl groups of
substances dangerous to health that should be eradicated. The Geneva/Aarhus Protocol seeks to control, reduce or eliminate discharge, emissions and losses of persistent organic pollutants in Europe concerning insecticide, fungicide, and rodenticide. Furthermore Ramsar convention 1971 on “wise use” of wetlands calls for sobriety in shoreline resource managements.

2.2.6 Soil organic matter and nutrient dynamics

Soil is the biogeochemical transformation field, a nutritive ban supporting plant life with seed-banking potential, CO₂, O₂ and N element regulation in the atmosphere, and a sink to pollutants beside other functions. Soil organic material (SOM) predominantly in zone A and B of soil profile is basically defined by stable natural or inherent features related to soil forming factors, and the loss of SOM results in a reduction in soil quality (Larson and Pierce, 1991; Carter, 2002). The soil material retains nutrients by effecting cation and anion-exchange, increasing water holding capacity, improving infiltration rates and enabling aggregate stability in soils (Jones et al., 2005; Barancicova et al., 2010) as well as improving tilth in the surface horizons. At decomposition they create essential benign habitats for microorganisms, protists, fungi (Unterseher, 2007) facilitating biogeochemical cycles. SOM reduces negative environmental effects of pesticides, heavy metals and many other pollutants (Doran et al., 1996) reduces crusting, soil erosion and facilitates penetration of plant roots. Soils containing <2% of organic matter are considered depleted and require upgrading. Grassland and forest soils, which can contain relatively high amounts of organic matter, generally have more sand-sized organic matter than arable soils (Carter et al., 2002) which undergo frequent tillage. Frequent tillage in cropland affects SOM by altering soil moisture contents, aeration, temperature conditions and often characterized by a lower soil organic carbon content as compared to similar soils under native forest or grassland on the same soil type (Guo and Gifford 2002) qualifying forests and grasslands as better soil preservers especially those nearer to water regimes. Although through tillage organic matter in plant, animal or waste residues are returned into the soils; it affects soil properties, mycorrhiza, glomalin production, biodiversity (Sieverding, 1991; Borie et al., 2006; Wright et al., 2007; Alguacil et al., 2008) and may change stabilizing mechanisms of soil organic matter (John et al., 2005). Constant need to produce food crops that require frequent tillage can be supplemented by perennial multipurpose species like Moringa tree crops in beneficial symbiosis either by agro-/forestry or alley-cropping.
Fig. 3 (A) Erosion start-off point at precipitation and its impacts on vegetation in Lake Victoria basin, Kenya. (B) Off-load point of top soil organic material.

*Arbuscular Mycorrhiza Fungi*

2.3 Biology, morphology, associations and benefits

AMF phylum *Glomeromycota* (Schüßler, 2001), are obligate biotrophs responsible for P transfer to the root system in exchange of photosynthates ranging between 5 to 85% of C, depending on plant species (Treseder and Allen, 2002) especially in P deficient soils. These are organisms packaged in microscopic units (Allen, 2007) known to grow in soil pores down to 2 µm and even penetrate the rock matrix while individual hyphae may only be 2 to 10 µm in diameter (Staddon et al., 2003; Bornyasz et al., 2005). Glomeromycotan intracellular fungi form trunk hyphae (3–6 mm), fine arbuscules (0.8–1.5 mm) and vesicles (20–30 mm). The monomeric glycoprotein structures in hyphae are coated with glomalin, bound together by hydrophobic interactions (Nichols, 2003) forstering stable soil macro-/microaggregates >0. 25 mm (Tisdale and Oades, 1975; Tisdall and Nelson, 1979). Research trends in the Glomeraceae taxa categories representing natural classification based on molecular phylogenetic analyses at present are recognized as *Glomus macrocarpum* and
Claroideoglomeraceae of Glomeraceae family (Schüßler and Walker, 2010). Current knowledge in effective symbiosis facilitation integrates inoculum to increase microbial/mycorrhizal populations in planta, via rhizobial-mycorrhizal-legume symbiosis.

2.3.1 Rhizobial-mycorrhizal-legume symbiosis

In the tripartite rhizobial-mycorrhizal-legume symbiosis, there is synergism between the partners in that the scarcity of available P in soil ecosystems limits legume rhizobia establishment and N$_2$ fixation in the absence of AMF formation (Barea, 2005). Sufficient literature exists on impacts of this symbiotic synergy. Wander et al. (1994) studied three farming systems: (1) animal-based (cover crops and animal manure only), (2) legume based (cover crop only), and (3) conventional (N-fertilizer). Their results showed that the two organic systems had higher levels of microbial activity and more diverse species than the conventional system. Negi et al. (1989) showed interactions between mycorrhiza and NFB in increased growth and nutrition of the barley plants where mycorrhizal plants alone had no effects. Requena et al. (2001) showed inoculation with autochthonous AMF and with rhizobial NFB not only enhanced the establishment of key plant species, but similarly increased soil fertility and quality. The dual symbiosis increased the soil N content, organic matter, hydrostable soil aggregates and enhanced N transfer from N-fixing to non-fixing species associated within the natural succession.

2.3.2 Dark Septate Endophytes

Dark septate endophytes (DSE) fungal structures are steadily gaining attention in the biospheric food chain. DSE “mycorrhizal” (Jumpponen, 2001), are a group of ascomycetous microfungi characterized by melanized cell walls (Jumpponen and Trappe, 1998; Addy et al., 2005) with ability to use complex nutrient sources, suggesting facultative biotrophic and saprotrophic habits (Mandyam et al., 2010). These are fungi or bacteria occurring inside plant tissues symbiotically, currently identified in most tropical crops such as coffee (Posada et al., 2007). Although their functions have not been well understood, DSE like mycorrhizae include protection against plant pathogens (Wicklow et al., 2005). DSE are broadly classified as conidial or sterile septate fungal endophytes, reported from 600 plant species including those that have been considered non-mycorrhizal (Jumpponen and Trappe, 1998). Unlike AMF, DSE seasonal dynamics are unknown. Various fungal structures of DSE depend on precipitation events and plant phenology (Barrow and Aaltonen, 2001). They coexist with the AMF and have been isolated from many grasses and forbs (Jumpponen and Trappe, 1998).
2.3.3 Benefits of mycorrhizal associations

Mycorrhizal benefits to soils are several, ranging from volatilization of C, H, and O in organic matter decomposition, mineralization and immobilization of N, P, K, S elements, and synthesis of humic materials, modification of soil permeability, aggregate promotion and even detoxification of soil. In details, mycorrhizal roles are well documented. Dehne (1982), Filion et al. (1999), Lendzemo et al. (2005) recorded its ability to fight pathogens. Smith and Read (1996), Puente et al. (2004) showed microbials increase N, K, Ca, Zn or Cu bioavailability and uptake in plants at aridity, the latter noting that these were also thermo/halo-tolerant. Barea et al. (2005), Ouahmane (2007) showed that mycorrhiza strongly modified functional abilities of the soil microflora. Duponnois et al. (2005), Ostle et al. (2009), Bardgett et al. (2009), Paterson et al. (2009), Deyn et al. (2011) recorded improved mineral nutrition, especially P, enhanced growth in hosts as well as aided effective fine root exudation activities in soil respiration. Pritsch et al. (2004) documented that AMF assists in enhancement of plants resistance to biotic and abiotic stresses such as nutrient exchange in cases of competition in unfavourable soils, raises soil pH in acidity, increases soil moisture levels and regulates temperature, ideal in soil conservation. Augé et al. (2001), Hildebrandt et al. (2007), Medina and Azcón (2010) investigated the importance of AMF in cases of increased drought and heavy metal resistance, documenting improved nutrient absorption. Schütz et al. (2010) reported on soil enzyme activity in microbial activities which determines biodegradation of organic compounds passing through soil profile by remediating soils in bio-augmentation. Orlowska et al. (2008) similarly reported that external AMF hyphae can bind Ni, influencing the uptake of heavy metals by plants in bioremediation. Gadd (2005) however reported earlier that this action in soil remediation may also accumulate metals from soil components. Pagano (2011) consequently concluded that the sequestered elements in mycorrhiza may reflect a polluted environmental condition, acting as a bio-indicator. Whichever the case, AMF population is an important tool in restoration and sustainability of degraded ecosystems.
Moringa

2.4 Description, distribution, ecology and benefits

Moringa (class Moringaceae; genera Rosidae) is a monotypic tropical forest species, falling under “underutilized” plants of the tropics (Price, 2000). *M. stenopetala* is endemic to Kenya, Ethiopia and Somalia while *M. oleifera* is indigenous to the sub-Himalayan tracts of north-west India, Asia. It is currently distributed throughout the tropics and was introduced in East Africa from India at the beginning of 20th century. Both species are now cultivated throughout the Middle East and in the tropics. About 14 species have been identified, the main ones being *M. stenopetala* and *M. oleifera*. They are commonly known as “drumsticks”, “horseradish,” “and miracle tree” locally, owing to their multiple usages, morphological features or other appropriate descriptions. Moringa is known as the poor man’s meat by some locals in Philippines. It is normally the first vegetable that sprouts immediately after long droughts when all vegetation dries out. To the poor this is a great gift from God. Moringaceae is precisely divided into three groups or sections; Moringa, Donaldsonia and Dysmoringa (Verdcourt, 1985). However morphological developments categorize them in to “bottle,” “slender” and tuberous shrubs (Olson and Rosedell, 2006). The latter described these further morphologically as follows; Donaldsonia being the ‘bottle tree’ with up to four clades, commonly distributed in southern parts of Africa and Madagascar Islands. These are types of trees with characteristically swollen trunks and radially symmetrical flowers. The second category, Dysmoringa, of up to six species of tuberous shrubs and sarcorhizal trees, characterized by bands of confluent paratracheal parenchyma alternating with bands of libriform fibres (Olson, 2000) are those allocated for North and Eastern Africa with thick and fleshy tuberous roots. Finally the “slender” tree clades are the morphologically slender-shaped trunks distributed in the Indian subcontinent and the Arabian Peninsula habitats. These are Moringa species with tough roots and bilaterally symmetrical flowers.

Moringa plants are fast growing/regenerating deciduous tropical trees. They can achieve up to 3.5 meters within one year and a diameter of 20-40 cm at chest height, while *M. stenopetala* clade ranges between 6-12 m tall with a diameter of 60 cm at chest height (Foidl et al., 2001). *M. stenopetala*’s crown is strongly branched though brittle, with soft wood. The leaves of *M. oleifera* are formed by imparipinnate-rachis of about 3 to 6 cm long with 2 to 6 pairs of pinnules while *M. stenopetala* upto 5 pairs of pinnae and 3-9 elliptic to ovate leaflets per pinnae. Each pinnule has 3 to 5 elliptical leaflets in *M. oleifera*, relative to height and age of the species. The flowers are in axillary, drooping panicles 10 to 25 cm long according to
standard measures at field levels. The pods of Moringa are elongated capsules borne singly or in pairs, pendulous, triangular tapering at both ends. The pods range from 25 to 45 cm long and 1.8 cm wide. Each pod accommodates 16 seeds embedded in the pith. Reproduction is bisexual, somewhat irregular zygomorphic, parietal placentaion and tricolporate pollen grains (Perveen and Qaiser, 2009). The seeds of *M. oleifera* are round embedded in a hard nut while for *M. stenopetala* are oval, with tissue-like pith in a softer seed hull (Morton, 1991; Reyes, 2006). *M. stenopetala* is of eudicot clade, with typical epigeal germination and a very high growth rate at an earlier stage with a record growth in height registered at >60cm in 30 days (Knopf, unpublished), compared to *M. oleifera* with an average of 20cm. Moringa has erect trunk, and greyish bark. The bark of young stem of *M. stenopetala* has phloem fibres arranged in wedges separated by dilated phloem rays (Olson and Rosell, 2006) acting as natural armors in protecting interior tissues. The root morphology like most plants is sensitive to soil types. The tuberous part acts as food and water reserves typically adopted for survival in arid regions/desert ecosystems. The extended branches grow in a disorganized manner and the canopy is umbrella shaped (Foidl et al., 2001). From this study *M. oleifera* repeatedly showed triarchic tuberous rooting systems unlike *M. stenopetala* that remained consistently singly tap-rooted (Knopf, 2007).

### 2.4.1 Biofertilizers and biopesticides

Moringa biomass potential as pesticides/fertilizer and/or plant growth enhancer (MPGE) is noteworthy. As a pesticide/fertilizer, Moringa extract as foliar spray can be applied on other agricultural crops instead of synthetic agrochemicals. The extract is achieved by grinding young Moringa shoots and making liquid spray thereof. Field trials with Moringa extracts in Asia already showed tremendous harvest increases in enriching agricultural land. MPGE in powder form has exceptionally high N, P and K and other mineral components beneficial in soil mineral restocking. Moringa compound in seedcake form left over after the oil extraction process is also ideal for mineral replenishment in the soil with growth promoting effects (Foidl, 2001). The plant growth hormones can increase yields by 25-30% for nearly any crop (Foidl et al., 2001). MPGE unlike chemical fertilizers are eco-friendly, sustainable and affordable.

### 2.4.2 Bioremediation or phytoremediation perspective

Bioremediation refers to biotechnology of extracting or degrading soil pollutants using bacteria, fungi or plants while phytoremediation uses plants and associated microbials to decontaminate polluted soils. Moringa plant material is a remarkable biodegrader/
phytoremediator of polluted sites, soils and surface/groundwater systems. Agrochemical industries emit effluence that infiltrates soils and water columns affecting water and soil properties. Moringa’s lignocellulose enzymes in plant stems acquired from sawdust or plant residues are useful chemical filters in biodegradation. Using partially defatted *M. Stenopetala* and *M. oleifera* powder to remove Cd$^{2+}$, Zn$^{2+}$, Cu$^{2+}$, and Cr$^{3+}$, Sajidu et al. (2006) observed remarkable heavy metal sorption ability in Moringa by applying metal concentration of about 4 ppm, the extracts showed complete sorption for Cd$^{2+}$, Zn$^{2+}$, and Cr$^{3+}$ ions at pH above 7.8, 4.0 and 4.0, respectively, at a dose of 1.0 ml of sorbent in 9.50 ml of metal solution. Baskaran et al. (2009) confirmed plant-microorganism benefits of *M. oleifera* as effective in improving plant performance in germination, growth, yields and biodegrading soil pollutants in seedcake as well as sorption activities in soil solutes. Mataka et al. (2010) investigated the potential of *M. stenopetala* and *M. oleifera* for Cd removal. With an initial Cd concentration of 7 mg/l, *M. stenopetala* seed powder, at a dose of 2.50 g/100 ml, this study group observed that Moringa powder reduced the concentration of Cd by 53.8%. Price (2000) similarly noted that one 100 kg of Moringa kernels can produce about 1 kg of pure polyelectrolyte that neutralizes the colloids as non-toxic natural polypeptides. Kalogo et al. (2001) used Moringa seed solution to enhance start-up of a self-inoculated upflow of anaerobic sludge blanket reactor in water treatments. They documented a significant biodegrader operation of Moringa with chains of benefits: (1) The solution shortened the biological start-up period by 20%, (2) increased acidogenic and methanogenic activity by a factor of 2.4 and 2.2 respectively, (3) increased the specific biogas production by a factor of 1.6, (4) favoured fast growth of the sludge bed, and (5) allowed the aggregation of coccoid bacteria and growth of microbial nuclei, which are precursors of anaerobic granulation. The plant polyelectrolyte is important in sedimenting mineral particles, fibres and organics in the purification of drinking water, cleaning vegetable oil and many other industrial uses (Sutherland et al., 1990; Sabale et al., 2008).

### 2.4.3 Other plant material benefits

The wood pulp extracts is high in lignocellulose content ideal in methanol production for biofuels for industrial and domestic use. A single mature seed in Moringa is approximately 40% oil in contents. Moringa oil is known to be of excellent quality (73% oleic acid, similar to olive oil) applicable in heavy industry such as cosmetics, machinery lubricants, cooking oil, fuel for lamps and perfumes (Ferreira et al., 2008). Other benefits include valuable nutritional food value returns to human, livestock, fish, and poultry diets (De Saint-Sauyeur, 1991). Price (2001) investigated quality nutritional impacts.
on cattle, documenting a weight increase of 3-5 kg on a Moringa feeding program. Milk production in this experiment increased from 7 litres/day to 10 litres/day. Weight gain was 1,200 grams/day while his control variables, had only 900 grams/day. This is a significant increase in production. Moringa’s leaves are a source of multivitamins, used as vegetables, salads with essential vitamins like A, B and C, plus Ca$^{2+}$, Fe, flavonoids, alkaloids, phenolics, carotenoids, such as β-carotene and other antioxidants e.g. the E-type composed of γ-tocopherols (Sutherland et al., 1990; Ferreira et al., 2008) and other proteins important in health.

**Tab. 1** Mineral content of Moringa plant material adapted from *Moringa oleifera: Natural Nutrition for the Tropics* by Lowell Fuglie.

<table>
<thead>
<tr>
<th>Compound contents /100g</th>
<th>Pods</th>
<th>Leaves</th>
<th>Leaf powder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>86.9</td>
<td>75.0</td>
<td>7.5</td>
</tr>
<tr>
<td>Calories</td>
<td>26</td>
<td>92</td>
<td>205</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>2.5</td>
<td>6.7</td>
<td>27.1</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>0.1</td>
<td>1.7</td>
<td>2.3</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>3.7</td>
<td>13.4</td>
<td>38.2</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>4.8</td>
<td>0.9</td>
<td>19.2</td>
</tr>
<tr>
<td>Minerals (g)</td>
<td>2.0</td>
<td>2.3</td>
<td>-</td>
</tr>
<tr>
<td>Ca (mg)</td>
<td>30</td>
<td>440</td>
<td>2,003</td>
</tr>
<tr>
<td>Mg (mg)</td>
<td>24</td>
<td>24</td>
<td>368</td>
</tr>
<tr>
<td>P (mg)</td>
<td>110</td>
<td>70</td>
<td>204</td>
</tr>
<tr>
<td>K (mg)</td>
<td>259</td>
<td>259</td>
<td>1,324</td>
</tr>
<tr>
<td>Cu (mg)</td>
<td>3.1</td>
<td>1.1</td>
<td>0.57</td>
</tr>
<tr>
<td>Fe (mg)</td>
<td>5.3</td>
<td>7</td>
<td>28.2</td>
</tr>
<tr>
<td>S (mg)</td>
<td>137</td>
<td>137</td>
<td>870</td>
</tr>
<tr>
<td>Oxalic acid (mg)</td>
<td>10</td>
<td>101</td>
<td>1.6%</td>
</tr>
<tr>
<td>Vitamin A - B carotene (mg)</td>
<td>0.11</td>
<td>6.8</td>
<td>16.3</td>
</tr>
<tr>
<td>Vitamin B -choline (mg)</td>
<td>423</td>
<td>423</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin B1 -thiamin (mg)</td>
<td>0.05</td>
<td>0.21</td>
<td>2.64</td>
</tr>
<tr>
<td>Vitamin B2 -riboflavin (mg)</td>
<td>0.07</td>
<td>0.05</td>
<td>20.5</td>
</tr>
<tr>
<td>Vitamin B3 -nicotinic acid (mg)</td>
<td>0.2</td>
<td>0.8</td>
<td>8.2</td>
</tr>
<tr>
<td>Vitamin C -ascorbic acid (mg)</td>
<td>120</td>
<td>220</td>
<td>17.3</td>
</tr>
<tr>
<td>Vitamin E -tocopherol acetate (mg)</td>
<td>-</td>
<td>-</td>
<td>113</td>
</tr>
<tr>
<td>Arginine (g/16g N)</td>
<td>3.6</td>
<td>6.0</td>
<td>1.33%</td>
</tr>
<tr>
<td>Histidine (g/16g N)</td>
<td>1.1</td>
<td>2.1</td>
<td>0.61%</td>
</tr>
<tr>
<td>Lysine (g/16g N)</td>
<td>1.5</td>
<td>4.3</td>
<td>1.32%</td>
</tr>
<tr>
<td>Tryptophan (g/16g N)</td>
<td>0.8</td>
<td>1.9</td>
<td>0.43%</td>
</tr>
<tr>
<td>Phenylalanine (g/16g N)</td>
<td>4.3</td>
<td>6.4</td>
<td>1.39%</td>
</tr>
<tr>
<td>Methionine (g/16g N)</td>
<td>1.4</td>
<td>2.0</td>
<td>0.35%</td>
</tr>
<tr>
<td>Threonine (g/16g N)</td>
<td>3.9</td>
<td>4.9</td>
<td>1.19%</td>
</tr>
<tr>
<td>Leucine (g/16g N)</td>
<td>6.5</td>
<td>9.3</td>
<td>1.95%</td>
</tr>
<tr>
<td>Isoleucine (g/16g N)</td>
<td>4.4</td>
<td>6.3</td>
<td>0.83%</td>
</tr>
<tr>
<td>Vaseline(g/16g N)</td>
<td>5.4</td>
<td>7.1</td>
<td>1.06%</td>
</tr>
</tbody>
</table>

Many other observations have been documented on the nutritional values of Moringa in the past. Ramachandran (1980), Ferriera et al. (2008) reported Moringa to be rich source of Ca$^{2+}$, K$^+$, waxes, quercetin and kaempferol, which are flavonoid compounds containing phenolic
hydroxyl with antioxidant potential and other important amino acids. They observed high content of \( \gamma \)-tocopherol ranging from 5.7\( \mu \)g/g (adult leaves) to 27.8\( \mu \)g/g (6 month-old leaves) of dry mass in whole plant study. Amaya et al. (1999) compared food value at 23000IU in Moringa with other vegetables e.g. broccoli, carrot, kale, spinach and lettuce and documented a difference at 5,000, 3,700, 2200, 1900 and 1000 of vitamin respectively in a comparative study. Other contents are the use of ascorbic acid, two important pharmacological alkaloids (moringine and moringinine), sympathomimetic group of bases (Dillard, 2000; Siddhuraju, 2003) important in medicine. The pterygospermin Moringa compound found in the flowers and roots, function not only for mass plant nutritional needs but possess powerful antibiotic and fungicidal effects on soil pollutants important to remediation activities (Makkar et al., 1997; Sabale et al., 2008; Ayssiwede, 2011).

2.4.4 Ecological and soil preferences

Moringa tolerates diverse growth conditions in most tropical climates ranging from arid/semi arid regions to moist/humid, monsoon climates. Ideal average annual rainfall reported so far is between 250 and 3000 mm. However Moringa requires minimal water to survive and failed to thrive in trails of Kakamega rainforest in western part of Kenya (Klopf, unpublished). In this region, Moringa had persistent root-rotting characteristics due to excess wetness. Moringa grows in altitudes from 600m to 1200m but can survive at 2000m above sea level and outlive light frosts (Reyes, 2006). Investigations show growing temperature of Moringacea ranges from 25-40°C thriving nearly in all types of soils apart from clay soils, but there is evidence of commendable plant performance of M. stenopetala observed ecologically in paddy “sticky cotton soils” (alkalinized clay particles of <1mm) with minimal aeration in Lake Victoria basin, Kenya in this study.

Moringa grows in a wide soil pH range from 5 to 9 but prefers slightly acidic soils (Reyes, 2006). Nel (2001) and Anwar (2006) reported changes in seed composition, relating this to drought events. Moringa’s root characteristics enable it outstand drought or stresses. Its fine roots existence is relative to soil types, meshier in sandy soils while coarser in paddy soils as earlier observed.

2.5 Moringa: Root architecture and plant competition

Architecture of root modules and life span depend on ecological factors related to soils and costs of root investments depending on growing conditions, competition, species (Watson, 2000; Hooker, 2000). The finest roots in plants like Moringa can be as coarse superficial
rooting systems functioning as fine roots as was observed (Knopf, 2007). Samples of Moringa fine roots species from Lake Victoria basin’s paddy soils (< 1µm aeration) measured up to around 2-3mm or more in diameter, no longer fine but rather coarser. Depending on plant species 30% of fine roots may live only up to a week while others a year or more (Wells and Eissenstat, 2001).

Root architecture development is important at plant competition and survival in stress. Plant competition requires the ability of plants to efficiently acquire resources shared by neighbours; interspecific (interspecies) or intraspecific. Effectiveness to compete determines plant fitness mostly at the face of stress such as drought (Chaves et al., 2005). In such situations symbiosis increases plant fitness and survival by assisting nutrient and water uptake (Auge, 2001; Medina and Azcón, 2010; Bellgard and Williams, 2011). According to Davidson (1969), the root mass determines the rate of absorption while leaf mass controls rate of photosynthesis in plants. Plants are known to optimise biomass partitioning strategies between roots and shoots, allocating more biomass to organs, acquiring the most limiting resource and adapting plant compartment size (Brouwer, 1983) especially at seedling establishments. This is necessary in ensuring vigour and convenient surface area at early stages in cases of weedy competitors Suzuki et al. (2002). Important parameters in root developments can be observed conveniently in rhizotrons.

**Rhizotrons**

Rhizotrons are non-destructive tools that allow dynamic individual root growth developments to be recorded (Gregory, 2006). The transparent windows with front screens and opaque covers shield light infiltration into the root system. Irrigation can be done manually, through porous polymer tubing (Dieffenbach and Matzner, 2000), micro-suction cups or tensiometers (Göttlein, 1999). After filling the rhizotrons with dried soil, the system must be rewetted and equilibrated. It is possible to observe threshold root formation in developmental stages using mirrors, cameras or videos connected to rhizotrons.

**2.6 Conceptual framework of study**

This thesis focuses on the effective ways of accommodating high P demand in the Kenyan native soils via facilitated symbiosis and possible integration of Moringa plant in low-input sustainable agriculture/forestation. Additionally, efficiency could be achieved by using
Moringa plant growth enhancers in restocking lost plant nutrients. Moringa mineral contents rank high in N, P, K, S, Fe and many other elements suitable for achieving required optimal plant nutritional needs.

The basis of this study was to test inoculum and soil potential in improving plant growth and establishment of tropical Moringa species in forestation or alley-cropping along 800-1800m gradient of Lake Victoria region, Kenya (Fig. 4). The concept is based on steering biological functions to enhance soil fertility in sustainable resource management.

**Fig. 4** Study design with selected soil types covering altitudes 800-1800 above sea level representative of Lake Victoria basin, and a possible reverse system flow by optimizing ecological plant-soil-microbial interactions.
3. MATERIALS AND METHODS

Sites and experimental approach

3.1. Study background

Fig. 5 Image of Lake Victoria basin showing vegetation cover and important landuse activities within the basin (Olago and Odada, 2003).

The study background was designed for Lake Victoria basin, covering 40 km transect stretch limit of Kenya side of Lake Victoria from the shoreline. The shoreline is currently 3,440 km in total covering an area of 68,800 Km$^2$. Multiple anthropogenic impacts e.g. deforestation, agriculture and resource use increase shorelines, allowing increased settlements and resource degradation. Over 90% of Lake Victoria basin is cropland (Fig. 5). Forest cover has been minimally reduced in favour of arable land.

3.2 Data Collection

3.2.1. Soil sampling, treatment and storage

Native soil sample choice was based on altitude differences (800, 1300 and 1800 meters above sea level) applicable to the lake. The choice was based on the main three representative
soil types inherent in the basin. The fourth type was a standard type (peat), which is a growth substrate, Biotpferde®. These were categorized according to landuse history/tillage frequency/altitude. Tillage frequency was identified as; high tillage (HT), medium tillage (MT) low tillage (LT) and (ST) standard substrate. The paddy LT frequency types were sampled from a rice growing district, Mwea, an irrigation scheme situated at (00° 42’ 00” N and 37° 22’ 00” E) and an altitude of about 1300m above sea level. These are large plantations served by two rivers, Nyamindi and Thiba. The rivers supply commercial plantations in the scheme with flood water for irrigation. For tillage, rotavators till the land up to 4 inches (10, 16 cm) deep. The farms are about 30,350 acres large. The rice fields are comparable to the rice fields in Lake Victoria basin in Ahero. The samples were chosen to acquire a more representative region cover.

The clay MT soil sampled from Chemelil, alfisol came from sugarcane farm in the area about 40 km from Kisumu within 00° 05’ 04.77” S and 35° 07’ 57.51” E location, an important water catchment area for Lake Victoria. The size of the farm is 5054 hectares of arable land and about 16171 hectares of continuous sugarcane plantations. The tillage systems are diverse ranging from heavy machinery tillage, mould ploughs down to small tills privately applied by farmers locally according to the size of the land. The land within the sugarcane plantations are seldom-tilled, need longer times to mature and can be harvested periodically using already established field stocks without further tills. Loam oxisol came from the humid highlands with high rainfall with an altitude of about 1800 m above sea-level. Although the soils are well-drained acidic-red loam of volcanic ash from the Rift Valley Volcanoes, it is an intensively cultivated region. The fields are frequently tilled using multiple techniques. Intensive tillage exacerbates soil erosion in the highlands. The soil coring points were from arable lands mostly used for annual crops but accommodate perennial cropping like tea bushes. The soils had been air-dried in the shade for two weeks, pulverised and passed through 2 mm size sieve to remove organic matter, transported to Germany stored in a laboratory at room temperature, analysed using VDLUFA MB I, D2.1 (Fingerprobe) A 5.1.1 (pH-Wert) and A 6.2.1.1 CAL-Methods, version 2002 in soil analyses methods. The soils had been used in an earlier study based in Helmholtzzentrum München, Soil ecology research institute, IBOE, of the Technische Universität München (TUM).
Tab. 2 Chemical characteristics of native soil types representative of Lake Victoria basin, Kenya.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Paddy</th>
<th>Clay</th>
<th>Loam</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic Carbon (% TM)</td>
<td>2.07</td>
<td>2.25</td>
<td>2.39</td>
</tr>
<tr>
<td>Total Nitrogen (% TM)</td>
<td>0.14</td>
<td>0.13</td>
<td>0.24</td>
</tr>
<tr>
<td>Nitrat-N (CaCl$_2$) mg/100g</td>
<td>3.04</td>
<td>1.48</td>
<td>1.07</td>
</tr>
<tr>
<td>Ammonium-N (CaCl$_2$) mg/100g</td>
<td>0.28</td>
<td>0.06</td>
<td>0.96</td>
</tr>
<tr>
<td>CaCO$_3$ (% TM)</td>
<td>&lt; 0.2</td>
<td>4.5</td>
<td>28</td>
</tr>
<tr>
<td>P (P$_2$O$_5$-CAL m) mg/100g</td>
<td>6</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>K (K$_2$O-CAL m) mg/100g</td>
<td>42</td>
<td>11</td>
<td>33</td>
</tr>
<tr>
<td>Cr (Dry mass) mg/kg</td>
<td>67</td>
<td>44</td>
<td>28</td>
</tr>
<tr>
<td>Cu (Dry mass) mg/kg</td>
<td>27</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Ni (Dry mass) mg/kg</td>
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</tr>
<tr>
<td>Calcium</td>
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<td>4.5</td>
<td>&lt; 0.2</td>
</tr>
</tbody>
</table>

* The standard soil with pH (CaCl$_2$):5, 5, KCl in g/l 1, 1 Solutes 150mg/l N, (CaCl$_2$) 150mg/l P$_2$O$_5$, Phosphate (CAL) 210mg/lK$_2$, potassium oxide (CAL) organic substance, 85% was used concurrently.

Fig. 6 Soil design selected according to tillage frequency; in standard (ST) or vermiculite, LT (low tillage), HT (high tillage), MT (medium tillage).
3.2.2 Seed collection, preparation and planting

Seeds from Kenya were acquired from Orongo village within co-ordinates (UTM) 699, 285.37 S and 9,986,154.13 E; 34° 45'/ 0° 00'; 0° 00'/ 35° 00'; 34° 45'/0 15'; 0° 15'/ 35° 00' and Kenya forestry research institute (KEFRI), Maseno within Lake Victoria basin. The first set of germplasm was developed from Moringa seeds grown in paddy “sticky black cotton soils” of vertisol and histosol origin. The second set had acidic loam oxisol background. The seeds were packed in plastic bags and transported to Germany for this study.

For pregermination steps, the seeds were dehulled and the nuts soaked in sterile water, for period 36 hours under room temperature in order to accelerate germination. The water was changed frequently to avoid fungal contamination. The seeds were planted in vermiculite initially and left to germinate before transplant into different soil types according to treatments and space. The treatments were divided into blocks based on soil design. The first block was purely native soils sampled from Kenya and a set of standard peat (growth substrate) or vermiculite. These blocks were set on a greenhouse bench with mean temperature of 20°C under ambient light supply, growth chamber with a constant temperature of 23°C and rhizotrons at room temperature. The seedlings were left to develop for two weeks before inoculation.

Greenhouse Experiments

3.3 Inoculum development

Cultured AMF propagules, *Glomus hoi* of University of York origin, registered at BEG, (BEG 104), BEG 68 (Berch and Trappe, 1985), *Glomus intraradices* (Schenck and Smith, 1982) and *Glomus mosseae* (Nicol. and Gerd; Gerd. and Trappe) in cocktail form was used to develop an AMF inoculum bank using *Plantago major* from open sources and were left to incubate for 2-3 days. The germinated seedlings were transplanted into 5x5x5 cm of 650 ml plastic containers. The containers were filled with autoclaved sand of 0.5-1mm size, collected from “Kronthaler Kieswerk,” Freising, Germany. The sand was washed several times with running tapped-water repeatedly to ensure cleanliness protocol; no contamination or other materials from the site. 2 g of inoculum was applied to each pot for about 15 pots in total for the cultures. These were irrigated daily using Hoagland and Arnon (1950) nutrient solution with 10 % of its original ionic force.
3.3.1 Experiment 1: Trap plant culture and harnessing of Mycorrhiza.
A biotest was done using 65 ml multipots with native soils that had been stored for long (4 years). Seeds of wild *Plantago major* were cleaned, dried and planted in 17 x 24 x 5 germinations trays in five parts of 5 x 5 of 15 trainers in each block. The seeds were sown directly into the soils. The blocks were left to establish in a greenhouse at an average temperature of about 20°C. From four to five days of germination, *Plantago major* plantlet images were taken to assess germination rates and plant performance. After two months, colonized roots were harvested for mycorrhizal assays. The rest of unharvested Plantago were left to further establish as inoculants for Moringa target-host plants in this study.

3.3.2 Experiment 2: Moringa seedling establishment in native soils and inoculation
Flexible Rootrainers® with open book design were used for seedling germination using vermiculite or standard as germination media before transplant. The deep rooting system oriented plastic containers allow continuous root growth development, observations and easy transplant with minimal disturbance. With clip-top design features, the ridged cells are held together allowing downward root architectural growth, avoiding tangling mass. Initially, plantago plantlets were used in evaluating mycorrhizal abundance in the soils before Moringa inoculation.

Moringa seeds (*M. stenopetala* and *M. oleifera*) were planted in 55cm³ size rootrainers of 195 total seedlings divided into 3 x 65 blocks of plantlets each in vermiculite for germination under a temperature of 23°C and a humidity of 70%. After two weeks of germination, the seedlings were transplanted into the native soils divided into four blocks according to soil types. Allochthonous versus autochthonous soil and inocula tests was separately done using paddy LT mixed with sand at ratio 2:1 (v/v). The seedlings were inoculated with AMF and NFB. Block 1→AMF inoculum, block 2→ AMF+NFB, block 3→harnessed autochthonous AMF and block 4→ AMF (*G. mosseae, G. intraradices* and *G. hoi*), termed as allochthonous mycorrhiza in this research.

3.3.3 Experiment 3: Moringa seedling establishment in standard soils and inoculation
In this experiment, 30 x 50 cm tray ideal in holding the trainers upright were applied. Moringa were directly planted into the standard soils without using pregermination media. *M. oleifera* and *M. stenopetala* established in a standard substrate with a pH of 5.7 were inoculated with a cocktail of *G. intraradices, G. hoi* and *G. mosseae* after two weeks of germination. One treatment and a control block of each species were set. Non-destructive sampling method
basal stem diameter and plant height) was used to record data on effects of inoculum on Moringa.

3.3.4 Inoculation of arbuscular mycorrhizal fungi and rhizobacteria
For all experiments Plantago major culture and chick-pea rhizobia (Rhizobium sp) of Cicer arietum legume was used to generate inoculum banks for Moringa inoculation. In all experimental blocks Moringa seedlings were inoculated after two weeks with the harnessed mycorrhiza or cultured AMF and rhizobacteria in legumes after transplant.

Growth Chamber Experiments

3.4 Inoculation and treatment of Moringa seedlings
3.4.1 Experiment 1: K probes on Moringa
A total of 195 of 55 cm³ volume rootrainers were arranged in trays divided into three blocks of 65 plantlets each. These were filled with vermiculite growth substrate. Moringa seedlings were directly planted in the substrate and left to germinate under a temperature of 23°C and a humidity of 70%. The seedlings were inoculated with AMF and NFB. After two weeks of germination K⁺ treatment was applied. The seedlings were irrigated with 2 mM of KCl salt solution for further two weeks.

3.4.2 Experiment 2: Moringa plant growth enhancer versus conventional fertilizer
Equal measure (30g each) of fertilizer (6% N; 20% P₂O₅ 4% K₂O, 1% MgO plus trace elements Fe, Cu, Mn, and Zn) and Moringa plant growth enhancer (MPGE), with natural chemical contents as described in Tab. 1, was applied on two weeks old Moringa seedlings established in vermiculite. The solute form of fertilizer was applied according to manufacturer’s recommendation (40g/10 litre dilution). A similar doze was used on seedlings with MPGE treatment and irrigated for a period of two weeks.

Rhizotron Experiments

In this experiment used 10 Plexiglas (Dieffenbach and Matzner, 2000) rhizotrons (cuvettes of 32 x 22, 5 x 1) cm) size was used to monitor root biomass developments. The rhizotrons were filled with native soils and a standard soil with the following details; pH (CaCl₂):5, 5 Salts, KCl) in g/l 1, 1 Solutes 150mg/l N, (CaCl₂) 150mg/l P₂O₅, Phosphate (CAL) 210mg/lK2,
potassium oxide (CAL) organic substance, 85%. 7 days old Moringa seedlings were transplanted into the rhizotrons after recording initial growth.

3.5 Irrigation and Soil Moisture Content (SMC) measurements
For the greenhouse experiments, irrigation was done via water pipe sprinkler once every day at the beginning of the experiment. The watering frequency was reduced after the seedlings were fully established. In the growth chamber irrigation was done consistently for the whole period of growth. The rhizotrons were watered manually according to need, 1 litre a day, directly according to need. For the water stress experiment, irrigation was withdrawn intervals of about 14 days before soil moisture measurements.

To test drought episodes Moringa plants were left without irrigation for weekly time intervals between SMC measurements. SMC in the rootrainers was assessed using time domain reflectometry (TDR; TEKTRONIX sensor, 1502 C/Tektronix Oregon, United States) according to Roth et al. (1990). A 12 cm head with two pins was installed in each root compartment for SMC reading.

3.6 Harvest: Greenhouse, Growth Chamber and Rhizotrons

Greenhouse samples
The plants in greenhouse were fully harvested at the end of the study period although non-destructive data collection of basal stem diameter (BSD) and plant height was done at intervals of six months over the period 2009-2011. A section of seedlings were fully harvested for biomass records. In all experiments, sampling protocol of basal stem diameter (Fig.7), plant height as well as biomass data were taken.

Fig. 7 Sampling point below cotyledon foliage of Moringa stenopetala (MS) and Moringa oleifera (MO).

Growth Chamber
Harvest was performed at different levels depending on data requirements within two months intervals for data uniformity for the sake of comparisons. Plant height and BSD were taken at two weeks intervals for at least a period of two months in each case. The seedlings were harvested for biomass measurements (fresh + dry weight) at the end of experiments.

**Rhizotrons**

At initial start of sampling, non-destructive method was done. Root tracings as well images of the root developments were taken and means calculated. Root growth parameters were measured at intervals according to treatments. At the end of the experiments, changes in root developments were recorded, the roots harvested and analysed, dry weight biomass taken.

3. 7 Identification, quantification and evaluation of mycorrhiza

The fine root aliquots were harvested according to soil types. The roots were washed carefully in running water. The 2 cm segments of fine roots were cut from the root mesh and further sorted into at least Ø 2mm sizes for microscopy.

**Staining of mycorrhiza and root segment microscopy**

Root segments were soaked in KOH 10% at room temperatures between 18-22°C. When the roots were still pigmented, alkaline H₂O₂ was made by adding 3 ml of NH₄OH to 30 ml of 10% H₂O₂ and 567 ml of tap water according to Brundrett and Abbott (1994) was applied. KOH solution was removed and HCl applied for 1-2 hours to neutralize alkaline KOH. The cleared roots were dipped in ink and vinegar according to Vierheilig (1998) for 12–24 hours at room temperature. This method was chosen not only due to its simplicity, but its predissolved state which enables omission of acidification steps as in other procedures. This accomplishes the bulk of samples in a shorter time. For optimum staining, different concentrations levels were used to achieve quality assessment. To improve the results, destaining in lactoglycerol was applied on the root samples. The stained segments were mounted on slides for microscopy.

A powerful light microscopy (Aristoplan®) connected to KAPPA® digital camera to detect very fine features of mycorrhization. For reliable data, light microscopy was used to identify morphological features of mycorrhiza in the stained fine root segments. Morphological criteria according to Brundrett and Abbott (2004) were used to define anatomical key mycorrhizal features.

**Scoring of Arbuscular Mycorrhiza Fungi**
AMF scoring procedure was done according to Trouvelot et al. (1986) where abundance was based on mycorrhizal structures such as intraradical vesicles, hyphae, and arbuscules and coils presence.

![Fig. 8 Quantification and evaluation of AMF colonisation of root segments according to Trouvelot et al. (1986).](image)

### 3.8 Measurements, calculations and statistical tests

The rhizotrons were harvested after two months each at intervals. During and after the experiment images were taken at different growth stages. The roots were processed using Epson® Expression scanner and stored in JPG file formats. Repeated data images of morphological root developments related to nutrient uptake were analysed using data processor WinRHIZO® (Regent Instrument, Quebec, Canada), at a resolution of 300 dpi with area targets of 1.00 cm² image parameter on thresholds, designed for colour and grey images. The threshold and colour calibrations were done. Colours were defined according to belowground, aboveground and background classes, defining exclusion regions for precision.

In cases of uncertainty, manual pencil tracing could be applied especially for the parts the camera may not detect. Parameters of calculations were based on the formulas below:

- **Fine Root Length (FRL) [m]** defined as length of a group of rootlets (root cohort) at time of sampling:

  
  Specific Root Length (SRL) [cm g⁻¹] → fine root length / fine root biomass

- **Specific Root Surface Area (RSA) [cm² g⁻¹]** → fine root surface area / fine root biomass

- **Specific Root Volume (SRV) [cm³ g⁻¹]** → fine root volume / fine root biomass

- **Root Tip Frequency (RTF) [n cm⁻¹]** → number of fine root tips / root length
Relative Growth Rate (RGR) → a measure for fine root production within a period of time. Parameters on focus were root diameter distributions, expressed in root-length volume, and biomass. Fine roots from rhizotrons were divided into Ø = 1 - 3 mm for mycorrhizal analyses. Dry and fresh weight were collected and recorded although for accuracy, only dry weight biomass was used in analyses. The total biomass for all blocks was weighed, oven-dried at 65, for 48 days. Two growth seasons were analysed according to Hunt (1982);

\[ RGR = (DW2 - DW1) \times DW3 \times (t2 - t1) \]

Where \( DW_1 \) is the dry weight at time point 1, \( DW_2 \) the dry weight at time point 2, \( t_1 \) the time point (days before start of treatment). The % relative growth rate was expressed in grams. The images were taken after cotyledons defoliation (Fig. 7).

Parameters for root architecture and growth analyses were done based on root mass, total biomass dry weight, basal stem diameter, and plant height used in evaluating effects of soils or treatments on Moringa.

The root:shoot ratio was used in analysing the ratio of the root biomass to the above-ground biomass in:

\[ R = \frac{M_{\text{root}}}{M_{\text{ag}}} \]

\( M_{\text{root}} \): dry root biomass
\( M_{\text{ag}} \): total shoot dry mass

Once a suitable, experimental dataset was identified, plant, soil and treatment data gathered, simple statistical tests were used. GenStat 9th Edition for windows (VSN) International Ltd., U.K.). Data were assessed for homogeneity of variance and normality through analysis of the residuals and posthoc comparisons of means for at least two months old Moringa seedlings. Linear regression model was applied in a two-way analysis of variance (ANOVA) no blocking, based on soil and inoculum effects on basal stem diameter, height increments for a more robust data, to ascertain if there were significant differences between soils and treatments. Differences were considered significant if \( P < 0.05 \).
CHAPTER FOUR

4. RESULTS

*Greenhouse Experiments*

4.1 Growth and plant response to inoculum

The first experiment was aimed at testing soil effects (paddy low tillage-LT; clay medium tillage-MT; loam high tillage-HT and a standard substrate) on plant performance, set under mean temperature of 20°C and ambient light growth conditions. A biotest was necessary to revitalize native soils and harness autochthonous mycorrhiza. *Plantago major* in this case, functioned as a plant performance indicator, trap culture and AMF inoculant for Moringa seedlings.

4.1.1 Effects of soil on *Plantago major* as inoculant and plant indicator

A higher germination of 62.5% in paddy LT compared to other native soils (33% and 58%) in total squares per tray was realized (Fig. 9; Plate A1). Growth and biomass records were higher compared to clay MT and loam HT. However a combination of loam HT soil type and vermiculite improved plant performance at 58% compared to HT soil type without vermiculite, registering 33%. Clay MT soil had delayed germination with slower growth rate comparable to paddy LT while standard (ST) and vermiculite had ≥90% germination rate.

![Germination Graph](image.png)

**Fig. 9** Performance of *Plantago major* planted in different soil types; standard (ST), paddy LT, loam HT and clay MT under greenhouse multi-pot culture. (Σplant/pot⁻¹).
4.1.2 AMF probes in native and standard soils (HT, MT, LT and ST)

*Plantago major* rootlets were used in evaluating degree of mycorrhizal colonization. The three native soil types showed different levels of mycorrhizal colonization intensities (Plate A2). Paddy LT samples exhibited high mycorrhizal presence in the soils observed in rootlet bioassays. Within two months up to 40% vesicle cover could be identified. With time, intensive AMF colonization characterized by the presence of mycorrhizal structures in the cortical cells was evident. The assays in the hyphopodium revealed AMF structures, mostly of *Glomus tenuis* clade. Tree-like intracellular arbuscules in ‘Arum-type’ infection units, AM hyphae, appressoria, arbuscules and intracellular hyphal coils in ‘Paris-type’ mycorrhiza were quantified. Rootlets sampled from paddy LT soils had higher degree of mycorrhizal colonization.

4.1.3 Seedbanking and biodiversity: Impacts on plant performance

The native soils revitalized at simple irrigation with unexpected high degree of plant community seedbanking potential. However plant diversity intensified competition effects on Moringa growth developments with significant impacts. At the beginning of this experiment in 2009, phase I (Figs. A4-7), both Moringa species displayed vigour and fast growth influenced by either soil properties or inoculum (AMF, NFB). With increased plant competition in phase III, negative growth response was recorded among Moringa seedlings, although inoculated seedlings still survived the stress. At weed control, a positive feedback on growth was realized immediately. Most Moringa seedlings lost all aboveground biomass, retaining only live tubers in most native soils apart from paddy LT and standard soils at depletion phase III. The greater the canopy covers from weedy competitors, the lower the target plant performance. Most abundant mycorrhizal features observed on the rootlets of weedy competitors were the hyphal strands which were frequently septate. A decrease on Moringa performance at Phase II indicated by yellowing and foliage loss was the initial signs of resource depletion phase, exacerbated by plant competition. Nevertheless positive response in growth parameters (BSD diameter and height) was still evident on inoculated seedlings at phases I and II. Most Moringa seedlings at Phase III remained belowground until regeneration or replant.
4.1.4 Effects of inoculum on Moringa seedlings

**Fig. 10** Development of basal stem diameter and height of *Moringa stenopetala* (MS) and *Moringa oleifera* (MO) plants relative to treatments (AMF and NFB). The seedlings were established in native (HT, MT, LT) and standard (ST) soils under ambient light in greenhouse. Data collected at different time frames from 2009-2011. (Means ±SD; n=6).

Overall mean BSD and height of Moringa growth parameters sampled in 6 months intervals from 2009 to 2011 were analysed (Figs. 10; A4-7). Changes on Moringa growth characteristics were realized, relative to soil and inoculum effects. Inoculation induced growth in height and BSD of Moringa. There was more active growth in both species at the beginning of sampling period in 2009, of 2 months old seedlings compared to latter stages. Inoculated Moringa grew faster than controls. Greater heights were achieved with either single or dual inoculation. AMF inoculated Moringa seedlings revealed higher BSD increments, compared to non-inoculates. Dual inoculation (AMF+NFB) constantly gave higher values in BSD and heights, compared to AMF alone or controls. Special was the performance in standard and
paddy LT soils with additional soil influence. Although inoculum promoted growth on *M. oleifera* and *M. stenopetala*, soil factor equally gained importance. In all blocks, apart from the depletion phase III in 2010, Moringa had relatively larger mean BSD and greater heights. Plants grown in paddy LT and standard soils had larger BSD values compared to loam HT and clay MT samples. AMF inoculated samples were slightly shorter than dual (AMF+NFB) inoculated seedlings and lost leafy biomass more easily. Faster growth rates and plant vigour were recorded in inoculated Moringa seedlings, although it was not clear whether growth rates were influenced by plant competition or mycorrhizal effects initially. Inoculated *M. oleifera* and *M. stenopetala* showed greater mean heights compared to control sets without inoculum in sampling period 2009-2011. Dual inoculum (AMF+NFB) on Moringa revealed higher performance than AMF singly or non-inoculated plants. In almost all cases *M. stenopetala* showed positive responsiveness to inoculum.

![Fig. 11 Biomass distribution of Moringa stenopetala (MS) and Moringa oleifera (MO) grown in paddy LT soils under greenhouse conditions sampled from 2009-2011. (Values are means of dry weight; n=3).](image)

Considering dry weight biomass distribution (Figs. 11-12), treatments influenced growth of *M. stenopetala* unlike *M. oleifera*. Dual inoculum (AMF+NFB) was again better than single or non-treated seedlings with greater heights and larger BSD. AMF+NFB inocula application stimulated *M. stenopetala* and *M. oleifera* biomass increments. AMF+NFB combination likewise induced larger tubers compared to control samples. Leafy biomass did not show
response to inoculum. *M. stenopetala* seedlings seemed to respond more favourably to inoculum compared to *M. oleifera* in all parameters. However better performance range was between AMF and NFB combined treatments in both species. Compared to plants established in standard substrates or vermiculite, samples grown in native soils consistently weighed lesser.

**Fig. 12** Influence of arbuscular mycorrhizal fungi (AMF) and nitrogen fixing bacteria (NFB) on total dry weight biomass of *Moringa stenopetala* (MS) and *Moringa oleifera* (MO) established in paddy LT. Box range 25-75th and 50th percentile median. Whiskers indicate highest and lowest values. (Significant α=0.05).

### 4.1.5 Effect of inoculum on soil moisture and drought episodes on Moringa

TDR soil moisture control technique had been applied on Moringa plants at intervals. Soil moisture content was used to analyse plant water stresses under greenhouse experiments in native and standard soils. Soil water content reduced with time of irrigation withdrawal but with minimal influence on Moringa performance. Inoculated plants showed higher leafy
biomass compared to non-inoculated ones. Interesting was plant behaviour at inoculation, where samples exposed to drought maintained foliage cover especially in standard soils. Non-inoculated samples did not regenerate in the whole period of stress and notably took longer to show tiny leafy symptoms.

4.1.6 Relationship between plant height and basal stem diameter on Moringa growth

![Graphs showing relationship between plant height and basal stem diameter.](image)

**Fig. 13** Relationship between basal stem diameter (BSD) and plant height of AMF inoculated *Moringa stenopetala* established in native HT, MT, LT and standard (ST) soils. Bi-directional error-bar analysis from 2009-2011. (Significant α=0.05). Ratio 1:2 diagonal intersection at vertical line x=5 and y=10.
Fig. 14 Relationship between basal stem diameter (BSD) and plant height of AMF+NFB inoculated *Moringa stenopetala* established in native HT, MT, LT and standard (ST) soils. Bi-directional error-bar analysis from 2009-2011. (Significant α=0.05). Ratio 1:2 diagonal intersection at vertical line x=5 and y=10.

Suppressed class: clusters at X ≤5
Fig. 15 Relationship between basal stem diameter (BSD) and plant height of non-inoculated Moringa stenopetala established in native HT, MT, LT and standard (ST) soils. Bi-directional error-bar analysis from 2009-2011. (Significant α=0.05). Ratio 1:2 diagonal intersection at vertical line x=5 and y=10.

Relationship between BSD and height developments in M. stenopetala was analysed using bi-directional error bars. Growth parameters were classified into two levels of performance i.e. expected and suppressed classes, where suppressed samples clustered below x ≤5 boundary in the scatter plots (Figs. 13-18). M. stenopetala data cluster often patched on the right side of the performance boundary, although with double shifts from the standard soil impact at phase II and IV of the control block. There was a strong correlation between treatments and growth of Moringa relative to soil factor. Inoculated samples clustered more on the expected side of x=5 compared to control blocks with more patches on the suppressed side or bordering the line. Larger deviations were registered in paddy LT and standard soil samples compared to other native soils. A similar trend was observed on height increments of inoculated M.
oleifera, where only inoculated plants at phase IV in 2011 regenerated. Effects of mycorrhizal and NFB inoculants on Moringa species subsequently facilitated faster growth and vigour, devoid of plant competition. Between phase I and II, inoculation improved growth as shown in BSD and height growth parameter analysis. The same situation was observed at phase IV where inoculates gained strength, consequently inducing greater heights and BSD. Samples established in Paddy LT or standard soils however survived this phase better than those in other native soils, although the seedlings mostly had defoliated shoots. Non-inoculated samples desiccated with no regeneration unless replanted (phase III). This pattern allowed competition as the only driver to the negative growth. A depression phase was realized in phase III on both species allowing larger clusters below suppressed boundary.

**Fig. 16** Relationship between basal stem diameter (BSD) and plant height of AMF inoculated *Moringa oleifera* established in native HT, MT, LT and standard (ST) soils. Bi-directional error-bar analysis from 2009-2011. (Significant α=0.05). Ratio 1:2 diagonal intersection at vertical line x=5 and y=10.
Fig. 17 Relationship between basal stem diameter (BSD) and plant height of AMF+NFB inoculated *Moringa stenopetala* established in native HT, MT, LT and standard (ST) soils. Bi-directional error-bar analysis from 2009-2011. (Significant $\alpha=0.05$). Ratio 1:2 diagonal intersection at vertical line $x=5$ and $y=10$. Suppressed class: clusters at $X \leq 5$.
Fig. 18 Relationship between basal stem diameter (BSD) and plant height of non-inoculated *Moringa oleifera* established in native HT, MT, LT and standard (ST) soils. Bi-directional error-bar analysis from 2009-2011. (Significant α=0.05). Ratio 1:2 diagonal intersection at vertical line x=5 and y=10.

From the scatter plot (Figs. 16-18), inoculated *M. stenopetala* registers clustered more patched on the right side of the boundary x ≥5, although the situation looked different in *M. oleifera* samples. The weight of sample cluster often fell on the suppressed right side of the boundary in treatments with a single shift experience on the standard soils at phase III of AMF inoculated seedlings in Phase II. Control samples patched heavily on the left side at Phase I and III while improvements were more evident at Phase II in standard and paddy LT soil variables. Soil and treatments were significant at p<0.001. Compared to *M. stenopetala*, *M. oleifera* showed more parallel intercepts in relationships. When compared with different treatments in all phases, a better picture of inoculum interactions with parameters could be assessed. Significant was the *M. stenopetala* and *M. oleifera* performance value shifts in non-inoculates. Unlike inoculated plants, majority of control samples did not meet the minimum performance target at the vertical line and were mostly considered suppressed. Compared to
non-inoculated Moringa, AMF or combined AMF+NFB inoculum generated clusters which occurred more on the right side of the boundary.

Tab. 3 Response of *Moringa stenopetala* and *Moringa oleifera* at inoculum in basal stem diameter (BSD in mm) from 2009 to 2011. Treatments are; AMF, AMF+NFB and control. (Means ±SD).

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</tr>
<tr>
<td>2011</td>
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<tr>
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<td>8.38</td>
</tr>
<tr>
<td>ST</td>
<td>19.00</td>
<td>6.43</td>
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Inoculants induced growth in Moringa indicated by increased heights and BSD (Tabs. 3 and 4). Moringa plants Inoculated with AMF+NFB caused larger deviations in the analyses. Although loam HT was the least in performance in all phases, it is important to note increases at inoculum, indicating treatments influenced growth. Mean differences in Moringa growth parameter values among clay MT, paddy LT and standard soil established samples was minor. In both species, combined treatments showed significant differences in plant growth analysis. A slight difference in performance was observed on *M. oleifera* although the difference between inoculated and non-inoculated samples was significant. A negative growth record was realized at phase IV, typical of Moringa in water or nutrient-related uptake fluctuations.

### 4.1.7 Interaction effects of soil types (ST, HT, and MTand LT) and treatments on biomass

Tab. 5 ANOVA; mean yields for *Moringa stenopetala* (MS) and *Moringa oleifera* (MO) set under different soils.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MO</th>
<th>MS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d.f.</td>
<td>m.s.</td>
</tr>
<tr>
<td>Height</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil</td>
<td>3</td>
<td>615,98</td>
</tr>
<tr>
<td>Inoc</td>
<td>2</td>
<td>2105,2</td>
</tr>
<tr>
<td>Soil vs inoc</td>
<td>6</td>
<td>132</td>
</tr>
<tr>
<td>Resid.</td>
<td>276</td>
<td>16,69</td>
</tr>
<tr>
<td>Totals</td>
<td>287</td>
<td>287</td>
</tr>
<tr>
<td>BSD</td>
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<tr>
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</tr>
<tr>
<td>Soil vs inoc</td>
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<td>1,788</td>
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<td>Resid.</td>
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<td>1,384</td>
</tr>
<tr>
<td>Totals</td>
<td>287</td>
<td>287</td>
</tr>
</tbody>
</table>

* Soils are classified into four types according to land use history (HT, MT, LT) except the standard soil type (Biotopferde ®). Treatments include; AMF+NFB, AMF inoculum and a control without inoculum.
Fig. 19 Interactions between (A) soil types, 1-4 (loam HT, clay MT, paddy LT, standard) and (B) treatments 1-3 (AMF, AMF+NFB, control) on basal stem diameter/root collar diameter and height increments of *Moringa stenopetala* (MS) and *Moringa oleifera* (MO). Box range 25-75th and 50th percentile median. Whisker represents highest and lowest values. (Significant α=0.05).
In ANOVA (Fig. 19; Tab. 5), interaction effects of soil and inocula (AMF+NFB) on heights of Moringa species grown under greenhouse was tested. Treatments impacted BSD developments of Moringa species. Soil factor had effects on Moringa growth in height and BSD development. Large variations were realized in *M. oleifera* height values compared to *M. stenopetala*, an independent variable. At regeneration phase I, all levels of treatments showed growth improvements induced by inoculum and soil factors. Soil and inoculum affected plant heights significantly (P <0.001). Effects of soil and inoculum interactions were significant to height increments of *M. oleifera* species unlike *M. stenopetala*. Whereas soil factor alone neither influenced BSD growth of *M. oleifera* nor *M. stenopetala* at P=0.539 and P=0.094 respectively inoculum singly had significant effects on BSD on Moringa.

4.1.8 Interactions of AMF cocktail (*G. intraradices, G. hoi, G. mosseae*) on Moringa plant growth in standard soils

![Graph showing interactions of AMF cocktail on Moringa plant growth](image)

**Fig. 20** Influence of *G. intraradices, G. hoi* and *G. mosseae* cocktail on (a) basal stem diameter and (b) height of *Moringa stenopetala* (MS) and *Moringa oleifera* (MS) grown in standard soil under ambient light in greenhouse. (Means ±SD; n=6).

AMF cocktail inoculates grown in standard soils had greater heights relative to non-inoculated samples. Special was *M. stenopetala* which had significant growth increments and vigour in comparison to *M. oleifera* in heights and BSD. There were no significant effects on AMF cocktail treatment on height of *M. oleifera* however inoculum induced BSD and height increments of *M. stenopetala*. Mycorrhized Moringa had larger BSD and greater heights compared to the non-inoculates.
4.1.9 Mycorrhizal colonization of Moringa root samples

Tab. 6 Estimates of mycorrhizal presence and abundance quantified among greenhouse experiments using light microscopy on plant rootlets, sampled from native and standard soil types. *)

<table>
<thead>
<tr>
<th>Colonization intensity %</th>
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<th>Phase II-2010</th>
<th>Phase III-2011</th>
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<td>MT</td>
<td>LT</td>
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<tr>
<td>Extra radical mycelium</td>
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<td>○</td>
<td>●</td>
</tr>
<tr>
<td>Auxiliary Cells</td>
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<td>○</td>
<td>○</td>
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<tr>
<td>Appresorium</td>
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<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Coils</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Hyphae Intercellular</td>
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<td>○</td>
<td>●</td>
</tr>
<tr>
<td>Vesicles</td>
<td>○</td>
<td>○</td>
<td>●</td>
</tr>
<tr>
<td>Arbuscles</td>
<td>○</td>
<td>○</td>
<td>●</td>
</tr>
<tr>
<td>Dark septate endophytes</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
</tbody>
</table>

*) ○ ≤ 0% ● ≤1% ●● ≤10% ●●● ≤20% ●●●● >50% ●●●●● >90%

AMF quantification

Quantification of autochthonous mycorrhizal abundance at different phases showed mycorrhizal accumulation dependant on time and soil types relative to different tillage and
landuse history (Tab. 6). The native soils indicated different colonization patterns according to types and landuse/tillage frequency. At phase I, trap culture analysis showed only paddy LT type of soil was more colonized with AMF. Mycorrhizal structures characterized by appresoria, coils, vesicles, hyphae arbiscules were observed in rootlets established in native soils (Plate A2). The loam HT and clay MT rootlets had mostly runner hyphae. Abundant DSE colonization presence was quantified. In standard soils, AMF inoculated samples showed a slower discriminate colonization degree despite low nutrient contents in soils. At phase II, colonization in paddy LT was still higher compared to other soils, apart from DSE hyphal strands and resting spores. Ornamented, pigmented, clustered or freelance DSE with vigorous hyphae, colonized root segments of weedy competitors (Plate A3). At phase III, >90% colonization degree of arbuscle quantification was realized from rootlets sampled from paddy LT soils. Other soils had lesser mycorrhizal structure presence. The weeds were clearly taller and vigorous than Moringa especially those of bidens family at the beginning of the third phase. M. stenopetala and M. oleifera showed discriminate colonization patterns compared to weedy competitors.

4.1.10 Autochthonous versus allochthonous mycorrhiza

Fig. 21 Soil and inoculum interaction effects on basal stem diameter increments of Moringa stenopetala. Treatments: NFB, AMF cocktail (G. intraradices, G. hoi and G. mosseae) on standard soils and harnessed mycorrhiza from native soils. Seedlings established in greenhouse experiments under ambient light. Different letters indicate significant differences, p<0.05. (Means ±SD; n=4).
Fig. 22 Soil and inoculum interaction effects on basal stem diameter increments of *Moringa oleifera*. Treatments: NFB, AMF cocktail (*G. intraradices*, *G. hoi* and *G. mosseae*), on standard soil and harnessed mycorrhiza from native soil. Seedlings established in greenhouse experiments under ambient light. Different letters indicate significant differences, p<0,05. (Means ±SD; n=4).

Fig. 23 Soil and inoculum interaction effects on height increments of *Moringa stenopetala*. Treatments: NFB, AMF cocktail (*G. intraradices*, *G. hoi* and *G. mosseae*) on standard soil and harnessed autochthonous mycorrhiza from native soil. Seedlings established in greenhouse experiments under ambient light. Different letters indicate significant differences, p<0,05. (Means ±SD; n=4).
Fig. 24 Soil and inoculum interaction effects on height increments of *Moringa oleifera*. Treatments: NFB, AMF cocktail (*G. intraradices*, *G. hoi* and *G. mosseae*) applied on standard soils and harnessed mycorrhiza from native soil. Seedlings established in greenhouse experiments under ambient light. Different letters indicate significant differences, p<0.05. (Means ±SD; n=4).

Indigenous versus cultured mycorrhizal effects on Moringa seedlings was analysed using paddy LT and the standard type, Biotopferde®. Harnessed inoculum in *Plantago major* was used in inoculation of Moringa species. Significant differences were realized between treated samples and controls (Figs. 21-24). Once more AMF inoculated plants were more vigorous and taller than non-inoculated ones. Samples that underwent dual inoculum (AMF+NFB) reasonably indicated faster growth. Larger BSD values were realized on seedlings established in standard soils compared to the paddy LT sample types. Autochthonous mycorrhiza seemed to have induced growth in *Moringa stenopetala*, but the effect was not evident on *M. oleifera*. Seedlings established in cultured AMF cocktail showed slightly larger BSD compared to harnessed autochthonous mycorrhizal effects from paddy LT soils. Although plant growth improvements were recorded in AMF + NFB treatments, autochthony showed mixed responses on height and diameter increment. Treatments improved plant growth and basal diameter sizes of Moringa plants promoting higher biomass. Samples inoculated with autochthonous mycorrhiza did better than AMF cocktail at initial stages of growth. In both *M. stenopetala* and *M. oleifera* autochthonous seemed to facilitate biomass increments (Fig. 25).
**Growth Chamber Experiments**

4.2 Moringa growth response to potassium, fertilizer, and inoculum treatments

The next objective was to assess differences in plant performance from the first objective in a follow-up experiment. Questions to answer were based on identifying factors responsible for biomass differences in the results. From soil analyses, paddy LT type had 42 per K (K₂O₅-CAL m) mg/100g content as compared to loam HT (11) and clay MT (33). Therefore K⁺ and mycorrhizal factor could be of significance in plant performance differences, warranting further specific tests. All experiments were set under controlled growth conditions of 70% humidity levels and temperature of 23°C.
4.2.1 K⁺ probes: Effects of inoculum (AMF + NFB) and K⁺ additions on Moringa species

**Fig. 26** Changes in height increments of two months old *Moringa stenopetala* (MS) and *Moringa oleifera* (MO) relative to arbuscular mycorrhizal fungi (AMF), nitrogen fixing bacteria (NFB), and potassium (K⁺) treatments. (Means ±SD; n=6).

**Fig. 27** Changes in basal stem diameter increments of two months old *Moringa stenopetala* (MS) and *Moringa oleifera* (MO) relative to arbuscular mycorrhizal fungi (AMF), nitrogen fixing bacteria (NFB) and potassium (K⁺) treatments. (Means ±SD; n=6).

Additional K⁺ test was performed on Moringa seedlings under growth chamber trials with improved growth conditions to give an insight into the factor responsible for plant performance differences in the experiment. *M. stenopetala* and *M. oleifera* was responsive to
treatment. AMF+K+NFB combination promoted larger BSD compared to other treatments in the row (Figs. 26-27). Moringa species showed insignificant height differences at treatments apart from AMF+K+NFB treated M. stenopetala. Considering vermiculite substrate as a variable, Moringa species in this block grew faster with more vigour than samples established in native soils. AMF inoculation increased plant heights in Moringa seedlings. However AMF+NFB inoculation once more yielded higher values compared to those without this treatment, irrespective of soil effects. Improved BSD and height increments in M. stenopetala and M. oleifera revealed positive plant responses at K element presence in combined treatments. The differences in mean heights and BSD were not so large among seedlings, although Moringa seedlings exhibited BSD and height increments especially at AMF+K+NFB combined treatments. Whereas most treatments caused slight improvements in height and BSD values in M. stenopetala, no significant differences were recorded on M. oleifera species.

**Fig. 28** Box plot of total dry weight biomass of Moringa stenopetala (MS) and Moringa oleifera (MO), with treatment levels, from T1-T4; AMF; AMF+NFB; AMF+K; AMF+NFB+K and T0 as control. Box range 25-75th and 50th percentile median. Whiskers indicate highest and lowest values. (Significant α=0.05).

A clear distinction was observed on Moringa dry weight biomass (Fig. 28). Treatment effects on total plant biomass showed higher values. Consistent with BSD and height increments, AMF+NFB+K application induced higher biomass records in both species. However AMF+K and AMF treatment was not significantly different, although a clear distinction between treatments and control plants existed.

**4.2.2 Root:shoot ratio of inoculated and potassium treated Moringa**
To assess resource allocation strategies and costs incurred on biomass production and longevity in Moringa, calculations on R:S ratio was performed. R:S ratio (Tab. A2 and A3) variations increased with treatments and soil types. While smaller variations could be observed on vermiculite established Moringa, larger R:S differences occurred on native soil samples of paddy LT. Treatment combinations AMF+NFB showed larger variations compared to single or non-inoculated plants. Similar to the seedling height and diameter, AMF, NFB and K⁺ treatments were highest in ratio. Although treated samples exhibited higher ratios, the differences were obviously not so large especially on *M. oleifera*.

### 4.2.3 Effects of commercial fertilizer and Moringa Plant Growth Enhancer

The third step in this research was focussed on the benefits of AMF and Moringa to Lake Victoria. N and P fertilizer are highly soluble and eutrophic. Former studies have analysed a combination of small amounts of fertilizer and inoculum as optimal ways of improving soil fertility regimes near water surfaces. However commercial fertilizers are expensive not just financially, but to environmental safety and life. An alternative to chemical fertilizer in sustainable resource management would be the application of the nutrient-rich Moringa plant growth enhancer (MPGE). Fertilizer experiment was therefore performed in order to evaluate plant performances comparatively.

![Graph](image.png)

**Fig. 29** Changes on plant height increments of *Moringa stenopetala* (MS) and *M. oleifera* (MO) at different treatment levels; fertilizer, nitrogen fixing bacteria (NFB), Moringa plant growth enhancer (MPGE), arbuscular mycorrhizal fungi (AMF), potassium (K⁺) and a control block, established in vermiculite. (Means ±SD; n=6).
Fig. 30 Changes on basal stem diameter of *Moringa stenopetala* (MS) and *M. oleifera* (MO) at different treatment levels; fertilizer, nitrogen fixing bacteria (NFB), Moringa plant growth enhancer (MPGE), arbuscular mycorrhizal fungi (AMF), potassium (K⁺) and a control block, established in vermiculite. (Means ±SD; n=6).

MPGE and fertilizer trials were set in vermiculite substrate under growth chamber. Treatments influenced growth rates, vigour and total biomass increments on *M. stenopetala* and *M. oleifera* (Figs. 29-30). *M. stenopetala* achieved greater heights at fertilizer or MPGE treatments compared to inocula. Special were combinations of FERT+NFB+AMF, MPGE+NFB+AMF, fertilizer or MPGE additions which promoted growth. Larger deviations in plant height were realized compared to narrower variations in BSD developments. While fertilizer and MPGE performance differed significantly on Moringa, combination AMF+K+NFB still showed strength in growth improvements. Plant height increment potentials improved with fertilizer or MPGE treatments, compared to K⁺ probe block. Fertilization promoted growth which was more evident on height developments contrary to BSD (Fig. 29). A modest change was observed on the treated samples compared to non-treated Moringa seedlings. Although fertilizer induced greater heights, MPGE similarly improved yields (heights and biomass) evidently, compared to other treatments. Apart from a similar performance between fertilizer and MPGE in *M. oleifera*, MPGE as an organic fertilizer enhanced root-tuber and BSD formation. From the experiment, treatments did not impact *M. oleifera* height or BSD increments. However, AMF+K+NFB treatment combination administered on *M. oleifera* showed slightly larger BSD at treatment compared to either fertilizer, MPGE or other treatments.
Fig. 31 Biomass changes in *Moringa stenopetala* (MS) and *Moringa oleifera* (MO) at different levels of treatment; fertilizer, nitrogen fixing bacteria (NFB), Moringa plant growth enhancer (MPGE), arbuscular mycorrhiza fungi (AMF), potassium ($K^+$) and a control block, established in vermiculite. (Means are values of dry weight; n=6).

Looking at the biomass developments, fertilizer treatments on *M. stenopetala* and *M. oleifera* improved yields (Fig. 31). There were differences in yields of *M. stenopetala* and *M. oleifera* compared to controls. Although BSD and height parameter value records were higher in fertilizer or MPGE treatments in Moringa seedlings, dry weight biomass increment differences among the two treatments were not so large. Once more inoculating Moringa with NFB and AMF improved growth significantly at MPGE or fertilizer additions. Inoculum induced growth, indicated by higher biomass in *M. stenopetala* and *M. oleifera*. The tuberous root biomass similarly showed higher values, however this development did not affect leafy mass of Moringa. In *M. stenopetala*, AMF+K+NFB treatment combination was again in consistency with effects of the same treatment on Moringa growth parameters. In *M. stenopetala*, there was no difference in AMF+K or AMF singly. The root tubers in *M. stenopetala* were noted to differ significantly at treatments contrary to *M. oleifera* which was evenly distributed. The tuberous roots of *M. stenopetala* were consistently heavier than aboveground dry weight biomass, compared to *M. oleifera*.
4.2.4 Effects of treatments on Moringa growth and root tuber developments

Fig. 32 Impacts of treatments on tuberous root diameter enlargements of *Moringa stenopetala* (MS) and *Moringa oleifera* (MO). Treatments involved; fertilizer, nitrogen fixing bacteria (NFB), Moringa plant growth enhancer (MPGE), arbuscular mycorrhizal fungi (AMF), potassium (K⁺) and a control block, established in vermiculite. Different letters indicate significant differences, p<0,05. (Means ±SD; n=6).

Moringa tuberous root development, like other growth parameters, was responsive to treatments. Fertilizer and MPGE induced larger tubers formation (Fig. 32) of Moringa species. *M. stenopetala* developed larger tuber diameters at little commercial fertilizer additions of upto 30mm, compared to control samples with 17, 6 mm. MPGE and fertilizer induced larger tuber formations although growth was not significantly different from K⁺ additions in the model. The samples established in commercial fertilizer and MPGE exhibited more fine rooting systems. MPGE addition into the soil influenced growth of *M. oleifera* compared to *M. stenopetala*, however both species showed more vigour and developed larger tuberous systems.
Fig. 33 Box plot analysis of total dry weight biomass on *Moringa stenopetala* (MS) and *Moringa oleifera* (MO) at different levels of treatments; fertilizer, nitrogen fixing bacteria (NFB), Moringa plant growth enhancer (MPGE), arbuscular mycorrhizal fungi (AMF), potassium (K+) treatments from T1-T8: (AMF; AMF+NFB; AMF+K; AMF+K+NFB; MPGE; FERT; MPGE+NFB+AMF; FERT+NFB+AMF) and a control, T0; established in vermiculite under growth chamber. (Significant α=0.05).

The box distribution (Fig. 33), revealed influence of treatments on Moringa growth and yield under controlled growth conditions. Inoculated (AMF and NFB), fertilizer, MPGE or K+ treated samples of *M. stenopetala* and *M. oleifera* showed biomass differences. There was a marked difference between treated and control samples in biomass increase, depending on levels of treatments executed on Moringa plants. Special were the changes on *M. oleifera* which revealed significant differences in biomass values. A better picture of treatment influence in growth chamber could be determined by observing the box behaviour in this experiment. A larger distribution was however realized on *M. oleifera* samples with higher yield. Unlike growth parameters (BSD and height measurements), treatment combination AMF+K+NFB showed a different picture. *M. stenopetala* showed a higher biomass turnover under this treatment compared to *M. oleifera* under the same treatment. Whereas fertilizer and MPGE grew faster and achieved greater heights, biomass records gave a different picture with lower values.
**Fig. 34** Box plot analysis of root and shoot dry weight biomass of *Moringa stenopetala* (MS) and *Moringa oleifera* (MO) at different levels of treatments; fertilizer, nitrogen fixing bacteria (NFB), Moringa plant growth enhancer (MPGE), arbuscular mycorrhizal fungi (AMF), potassium (K⁺); T₀ are control samples; T₁-T₈; AMF; AMF+NFB; AMF+K; AMF+K+NFB; MPGE; MPGE+NFB+AMF; FERT; FERT+NFB+AMF) established in vermiculite. (Significant α=0.05).

Above- and belowground biomass among treatments (Fig. 34) resulted in improved weights depending on levels of treatment administered. Unlike treatment combination AMF+NFB+K which showed a more skewed representation, fertilizer and MPGE added value to performance, with evenly distributed median in the analysis. Apart from faster growth, fertilizer and MPGE samples had greater heights comparatively. Little or no response was registered among other treatment blocks, where performance differences were minor. Fertilizer or MPGE effects on *M. oleifera* were more evident in yield improvements compared to *M. stenopetala*. A similar effect was however not observed in BSD developments in Moringa species.
Fig. 35 Relationship between basal stem diameter and plant height of two months old *Moringa stenopetala* seedlings at different treatment levels; fertilizer, nitrogen fixing bacteria (NFB), Moringa plant growth enhancer (MPGE), arbuscular mycorrhizal fungi (AMF), potassium (K⁺) and a control, established in vermiculite.

Growth rates and biomass turnover of Moringa depended on treatments administered on the seedlings. Developments of BSD and height parameters increased with treatments (Figs. 35-36). Whereas AMF, K and NFB seemed to improve performance, fertilizer or MPGE additions caused changes in growth characteristics of *M. stenopetala* and *M. oleifera* causing changes in values significantly. Additional AMF and NFB to fertilizer or MPGE plants stirred vigour compared to fertilizer or MPGE alone, but with higher performance than controls in
the experiment. Significant differences in heights of *M. stenopetala* and *M. oleifera* at treatments revealed that inoculum, MPGE and fertilizer fertilization stimulated BSD, plant height and biomass increments. With increasing BSD and height, a time-dependant biomass increase was evident.

**Rhizotrons**

Finally, it was necessary to analyse root growth parameters to help explain nutritional factors related to plant-soil-microbial interactions in a non-destructive method. Morphometric fine root parameters such as root growth rates, specific root lengths, surface area, root volumes, root tip frequency were of significance, in explaining mycorrhizal activities connected to nutrient bioavailability and uptake by plants. With increased mycorrhization, efficiency in nutrient and water uptake could be deduced as indicated by faster growth and plant vigour.

### 4.3 Fine root analysis: Effects of treatments on fine root developments

Tab. 7 Effects of treatments on Moringa root growth rate (% day$^{-1}$) of two months old (67 days) *Moringa stenopetala* and *Moringa oleifera* rhizotron samples in phases ($t_1$...$t_2$).

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<td><strong>[cm]</strong></td>
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<td>Verm+Inoc</td>
<td>165.2</td>
<td>240.0</td>
<td>67</td>
<td>14.528</td>
<td></td>
<td></td>
<td>2,17</td>
</tr>
<tr>
<td>Verm+MPGE</td>
<td>379.23</td>
<td>810.23</td>
<td>67</td>
<td>21.365</td>
<td></td>
<td></td>
<td>3,19</td>
</tr>
<tr>
<td>Verm+FERT</td>
<td>92.79</td>
<td>100.1</td>
<td>67</td>
<td>10.788</td>
<td></td>
<td></td>
<td>1,61</td>
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<tr>
<td>Vermiculite</td>
<td>47.54</td>
<td>90.60</td>
<td>67</td>
<td>19.58</td>
<td></td>
<td></td>
<td>2,84</td>
</tr>
</tbody>
</table>

*nd: data unavailable*
Fig. 37 WinRHIZO® screenshot of root system analyses of 1-5 days old seedling of *Moringa stenopetala*.

Data analysis from WinRHIZO® was divided into two blocks based on soil or inoculum treatments. It is important to note that within 24 hours root growth in *M. stenopetala* from 3 laterals to 37 in number (Fig. 37) was achieved. Both Moringa species had relatively higher growth rates. Fine root production activities changed with soil factor in dependance to time of growth. Mycorrhized rootlets on treated plants showed improved growth, explaining increased nutrient uptake compared to non-colonized roots.

At the second phase of rhizotron establishments, soils with self-regenerated organic matter in native soils. In these results, Moringa performed better than the first soil condition without organic matter. Comparatively, native soils had lesser root biomass yield but an improved performance in the soils at the second phase was revealed. Compared to phase I where foliage yellowed and fell more rapidly with lesser leaf regeneration, phase II and III showed much stability.
Fig. 38 Tracing of 7 days old Moringa root system (A) at the beginning of fine root measurements and image of fine root production of *Moringa stenopetala* (B); Image of two months old Moringa established in vermiculite at the end of sampling period.

At the beginning of growth period, the rhizotrons showed higher root growth rate compared to pots or roottrainers. Root production was faster on the inoculated rhizotrons compared to pot/roottrainer samples. The rhizotrons established in vermiculite had the highest root production (Fig. 38) while native soils had highest fine root loss after replant. Loamy HT soils, contrary to earlier results showed higher growth rates 25% (Tab. 7), while other native soil types ranked lower. Fine root increment from 176.90 to 217, 39 cm of MPGE was realized. This was comparatively higher than other treatments or soil variables although fertilizer again exceeded MPGE or single inoculum treatment in the row. Root tip production ranged from 12 to 681 and fork count number from 27 to 1955 was registered in the rhizotron grown inoculated Moringa. However paddy and clay native soils developed superficial root formation with lesser fine root count. A slightly higher root production was realized at inoculum in-put in vermiculite established plants; however the outcome of the native soil variables depicted change in growth related to soil factor.

MPGE treated plants in rhizotrons showed slightly higher values in heights, compared to fertilizer treatments. However root and leaf production in some cases did not show
In experimental years 2010 and 2011, root images were taken at different development periods to analyse morphometric changes of fine roots in *M. stenopetala* and *M. oleifera* at treatment administration. The seedlings had been exposed to different treatments as illustrated in Tab. 8. Root morphology adjustments to treatments were realized in both Moringa species where fine roots became finer in rhizotrons compared to pot/rootrainers. Roots planted in vermiculite or inoculated group in rhizotrons grew faster. Root lengths were modified by soil effects, reshaping root architecture of Moringa. A tendency towards higher average diameter (AvD) values was observed in treatments (inoculum or fertilizer) of Moringa. Higher biomass root production and finer root lengths were observed in rhizotron established *M. stenopetala* and *M. oleifera*. Although slight growth differences could be detected between 2010 and 2011 samples, minor changes were recognized in vermiculite samples. The average fine root

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**Tab. 8** Fine root growth analysis of *Moringa stenopetala* and *Moringa oleifera* at different growth periods from 2010-2011. Treatments involved; Fertilizer, MPGE, AMF and AMF+NFB. (Means; ±SE).

<table>
<thead>
<tr>
<th>Treatment</th>
<th><em>Moringa stenopetala</em></th>
<th><em>Moringa oleifera</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Root parameter</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2010</td>
<td>FERT</td>
<td>MPGE</td>
</tr>
<tr>
<td></td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>SRA [cm² g⁻¹]</td>
<td>46.87 ± 1.80</td>
<td>39.69 ± 1.65</td>
</tr>
<tr>
<td>AMF</td>
<td>25.62 ± 6.93</td>
<td>20.99 ± 8.36</td>
</tr>
<tr>
<td>SRV [cm³ g⁻¹]</td>
<td>7.23 ± 4.56</td>
<td>6.18 ± 2.93</td>
</tr>
<tr>
<td>RTF [n cm⁻¹]</td>
<td>0.52 ± 0.11</td>
<td>0.51 ± 0.19</td>
</tr>
<tr>
<td>AvD [mm]</td>
<td>5.62 ± 1.39</td>
<td>5.54 ± 1.43</td>
</tr>
<tr>
<td>AMF+NFB</td>
<td>SRA [cm² g⁻¹]</td>
<td>68.6 ± 4.34</td>
</tr>
<tr>
<td></td>
<td>73.65 ± 10.80</td>
<td>43.83 ± 1.79</td>
</tr>
<tr>
<td></td>
<td>11.75 ± 5.37</td>
<td>3.29 ± 1.71</td>
</tr>
<tr>
<td></td>
<td>0.57 ± 0.33</td>
<td>0.29 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>8.44 ± 7.95</td>
<td>6.21 ± 1.29</td>
</tr>
<tr>
<td>2011</td>
<td>SRA [cm² g⁻¹]</td>
<td>55.28 ± 18.16</td>
</tr>
<tr>
<td>AMF</td>
<td>37.36 ± 7.86</td>
<td>12.99 ± 8.74</td>
</tr>
<tr>
<td>SRV [cm³ g⁻¹]</td>
<td>6.51 ± 4.57</td>
<td>6.82 ± 2.94</td>
</tr>
<tr>
<td>RTF [n cm⁻¹]</td>
<td>0.3 ± 0.13</td>
<td>0.42 ± 0.19</td>
</tr>
<tr>
<td>AvD [mm]</td>
<td>5.62 ± 1.46</td>
<td>5.54 ± 1.43</td>
</tr>
<tr>
<td>AMF+NFB</td>
<td>SRA [cm² g⁻¹]</td>
<td>25.78 ± 45.49</td>
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<td></td>
<td>15.19 ± 10.95</td>
<td>37.69 ± 18.88</td>
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<tr>
<td></td>
<td>7.88 ± 5.51</td>
<td>17.08 ± 4.68</td>
</tr>
<tr>
<td></td>
<td>0.87 ± 0.34</td>
<td>0.59 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>6.21 ± 1.29</td>
<td>4.67 ± 4.68</td>
</tr>
</tbody>
</table>
Biomass (without mycorrhiza) of the fine rootlets inside the rhizotrons was lower than mycorrhized rootlets in the experiment. Treated Moringa showed slightly longer fine root lengths in analysis compared to controls.

**Tab. 9** Biomass distributions relative to soil factor and inoculum in rhizotrons from 2009-2011. Phase 1 of the experiment is set without soil organic matter (SOM); Phase II and III with regenerated SOM. Treatment; arbuscular mycorrhizal fungi (AMF). (Values are means of dry weight).

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Soils/Treatments</th>
<th>Leaves [g]</th>
<th>Stem [g]</th>
<th>Total root [g]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phase I_2009</strong></td>
<td><em>Moringa stenopetala</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moringa stenopetala</td>
<td>HT</td>
<td>0.2</td>
<td>0.1</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>MT</td>
<td>0.9</td>
<td>0.5</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>LT</td>
<td>0.2</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>ST</td>
<td>0.2</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>Verm+ Inoc</td>
<td>0.3</td>
<td>0.4</td>
<td>1.02</td>
</tr>
<tr>
<td></td>
<td>Vermiculite</td>
<td>0.1</td>
<td>0.2</td>
<td>0.8</td>
</tr>
<tr>
<td>Moringa oleifera</td>
<td>HT</td>
<td><em>nd</em></td>
<td><em>nd</em></td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>MT</td>
<td><em>nd</em></td>
<td><em>nd</em></td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>LT</td>
<td><em>nd</em></td>
<td><em>nd</em></td>
<td><em>nd</em></td>
</tr>
<tr>
<td></td>
<td>ST</td>
<td><em>nd</em></td>
<td>0.9</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>Verm+ inoc</td>
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<td>0.4</td>
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<td><strong>Phase II_2010</strong></td>
<td><em>Moringa stenopetala</em></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Moringa stenopetala</td>
<td>HT</td>
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<td>0.62</td>
<td>1.32</td>
</tr>
<tr>
<td></td>
<td>MT</td>
<td>0.56</td>
<td>0.65</td>
<td>1.74</td>
</tr>
<tr>
<td></td>
<td>LT</td>
<td>0.4</td>
<td>0.5</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>ST</td>
<td>0.2</td>
<td>0.4</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>Verm+ inoc</td>
<td>0.7</td>
<td>0.77</td>
<td>1.38</td>
</tr>
<tr>
<td></td>
<td>Vermiculite</td>
<td>0.52</td>
<td>0.82</td>
<td>0.44</td>
</tr>
<tr>
<td>Moringa oleifera</td>
<td>HT</td>
<td><em>nd</em></td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>MT</td>
<td><em>nd</em></td>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>LT</td>
<td><em>nd</em></td>
<td><em>nd</em></td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>ST</td>
<td>0.1</td>
<td>0.5</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>Verm+ inoc</td>
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<td>0.08</td>
<td>0.06</td>
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<td>Vermiculite</td>
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<td>0.03</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Phase III_2011</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Moringa stenopetala</td>
<td>HT</td>
<td>0.5</td>
<td>0.6</td>
<td>1.3</td>
</tr>
<tr>
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<td>MT</td>
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<td>0.27</td>
<td>0.11</td>
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<td>LT</td>
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<td>ST</td>
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<td>0.41</td>
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<td>Moringa oleifera</td>
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<td></td>
<td>MT</td>
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<td><em>nd</em></td>
<td><em>nd</em></td>
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<tr>
<td></td>
<td>LT</td>
<td>0.51</td>
<td>0.23</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>ST</td>
<td>0.4</td>
<td>0.32</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>Verm+ Inoc</td>
<td>0.3</td>
<td>0.37</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Verm</td>
<td><em>nd</em></td>
<td><em>nd</em></td>
<td><em>nd</em></td>
</tr>
</tbody>
</table>

* data not available
Samples established in rhizotrons exhibited faster growth with greater heights than seedlings planted in pots/rootrainers. The seedlings achieved up to 60cm in height within 5 weeks. However consistency with biomass measurement failed. High plant competitiveness for light was observed, consequently exhibiting higher growth rates and heights. *M. stenopetala* and *M. oleifera* grew faster at phase II compared to phase I. Phase II and III similarly had more biomass distribution (leaf, stem and root), compared to Phase I in native soils which were devoid of organic matter. Most *M. oleifera* seedlings lost leaf biomass at stress, apart from inoculated Moringa or seedlings grown in standard soils. Inoculated *M. stenopetala* established in vermiculite at Phase I did better than non-inoculated seedlings in rhizotrons, although the situation was different for *M. oleifera* seedlings which showed little responses at inoculation. The rhizotron-biomass records were remarkably lower than pots/rootrainers, especially the roots which suffered edge, space or competition effects. Most rhizotrons established in non-regenerated soil in phase I maintained live tubers belowground apart from paddy LT, contrary to greenhouse or growth chamber results while stability and growth improvements was clearer at II and III phase of rhizotron experiments.

![Graph](image)

**Fig. 39** Changes in basal stem diameter (BSD) of inoculated *M. oleifera* established in native paddy LT from 2009 -2011.
**Fig. 40** Changes in plant height of inoculated *M. oleifera* established in native paddy LT from 2009 -2011.

**Fig. 41** Changes in basal stem diameter (BSD) of inoculated *M. stenopetala* established in native paddy LT from 2009 -2011.
Mean height of Moringa plants increased depending on time and treatments applied in each block (Figs. 39-42). Regression equation had been used to show growth parameter increments in relation to treatments and soil factor. Mean annual increments suggesting an active growth trend at earlier stage in 2009 compared to the following years was observed. Although the differences in growth parameters between inoculated Moringa plants and control samples was significant at P<0.001, existing independent variables depicted differences that could be explained by other factors touching autochthony, soil properties, competition effects and other edaphic stresses that could be responsible for slower growth at some time points in Moringa established in native soils. Compared to typical development trends of Moringa established in vermiculite or standard substrates, native soil variables or treatments ranged within the limits of expected performance.

Paddy soils exhibited regeneration potential close to standard soils with diameter limits falling within the same range. Mean height and diameter increments were time dependant. Negated growth experience at the second sampling season in 2010* showed less inoculum response. In most cases mean differences were smaller across treatments, while dual inoculum did better than single inoculation in height and BSD growth.

**Fig. 42** Changes in plant height [cm] of inoculated *M. stenopetala* established in native paddy LT from 2009-2011.
CHAPTER FIVE

DISCUSSION

*Performance of Moringa Species*

5.1 Soil-plant-microbial interactions under greenhouse

Sieverding (1991), Gianinazzi-Pearson (2002), Ouhmahne (2005) Medina and Azcón (2010) have reported on the potential of mycorrhiza useful in improving degraded soils at aridity. In this study infertile native soils with heavy metal presence history, devoid of organic matter, could still be revitalized by simple irrigation and mycorrhizal application. Plants established in native soils with mycorrhizal abundance or inoculated Moringa plants revealed higher plant performance as a result of improved soil fertility enhanced by mycorrhizal activities, supporting these studies. Faster plant growth was perceived to be dependent on soil microbial population, resulting in improved nutrient availability, nutrient uptake and consequently accelerated growth (Fig. 10). The paddy low tillage (LT) mostly of histosols and vertisols origin, exhibited high germination rates, biomass increments and intensive AMF colonization. From the soil analyses (Tab. 2), paddy LT soils cored from of rice fields had notably higher AMF population and $K^+$ content compared to clay MT and loam HT native soils. From follow-up tests using plant growth and biomass parameters, both AMF and $K^+$ and AMF abundance seemed to have influenced plant growth under greenhouse experiments. The loamy HT soils mixed with vermiculite showed growth improvements however at stress, performance rapidly decreased. This could be due to lower mycorrhizal population verified in these soils.

Interaction between tillage and mycorrhizal population was found to be of significance. Plants established in loamy HT soils had minimal mycorrhizal colonization. Medium and low tillage soil classifications exhibited more diverse mycorrhizal structure formations, indicating AMF richness. In this case, tillage background probably interfered with AMF population supporting literature from Borie et al. (2006); Wright et al., (2007); Alguacil et al. (2008) on effects of tillage on mycorrhiza, biodiversity and soils. Looking at the plant biomass differences, tillage intensity correlated with AMF abundance, consistent with these reports. One of the possible reasons could be the stabilizing mechanisms of soil organic matter which changes with tillage (John et al., 2005) in frequently tilled soils. It is possible that other factors related to
mycorrhizal abundance such as soil properties could be likewise responsible for the differences in performances of Moringa in the native soils.

5.1.1 Effects of inoculum (autochthonous and allochthonous) on Moringa

Ouahmane (2007) observed that cultured *G. intraradices* seemed to promote growth of *Cupressus atlantica* compared to autochthonous mycorrhiza although by contrast, inoculated plants with the latter mobilized P from rock phosphates more efficiently than allochthonous *G. intraradices*. From the greenhouse results, autochthonous mycorrhiza seemed to promote growth but in a much slower rate of colonization (Fig. 23) compared to cultured AMF cocktail, consistent with this literature. Harnessed indigenous mycorrhiza and cultured AMF cocktail applied on Moringa species established in paddy LT and standard soil improved growth in *M. stenopetala* and *M. oleifera* considerably. Basal stem diameter and height values reflected improved plant nutrition at inoculum. It was not clear about the differences in performance in plants inoculated with autochthonous mycorrhiza and the cultured AMF cocktail, since performance indicators continued to alternate with time. This behaviour could be compared to Schreiner (2007), who observed that plant growth was not affected by either native or non-native *G. mosseae* isolates in one case, although in another experiment, non-native isolates were registered to be more effective in promoting growth and nutrient uptake compared to native ones. However caution needs to be taken when applying exotic cultured mycorrhiza ecologically due to risks of function shifts from mutualism to parasitism or even antagonistism (Bellgard and Williams, 2011). Soil factor is of importance when determining success of symbiosis beneficial to Moringa growth and prosperity. Mycorrhizal inoculum was found to be significant in yield increments, supporting (Puente et al., 2004; Barea, 2005; Schnepf et al., 2007; Atul-Nayyar et al., 2009) that inoculum significantly promotes plant nutrition and growth.

5.1.2 Mycorrhizal potential at plant competition

At greenhouse level, Moringa performance was affected by plant competition at phase III. In phase I and II, basal stem diameter and plant height was significantly improved at inoculation in all soil types. However at the beginning of phase II, Moringa performance decreased, indicated by yellowing and defoliation. Moringa seedlings maintained either <1 cm height dry mass at resource depletion phase III. Of these were few woody stems with dry tips in some pots, otherwise most plant material dessicated at stress, reducing biomass costs to live tubers only. Orshan and Geula (1962), Walter (1986) and van Damme (1991) described this process
of arid plants as 'survival by partial death' or 'partial drought evasion' through shedding the transpiring organs (mostly leaves and fine roots) at stress with recovery at the end of drought episodes.

Inoculating Moringa species however enabled plant yield improvement and sustained live tubers in the soil for regeneration possibility. Non-inoculated seedlings dessicated faster at phase III giving way to more aggressive weedy competitors. This implies that Moringa requires assisted symbiosis in order to compete effectively for limited resources. Since inoculated plants narrowly survived drought episode, it is important to increase mycorrhizal population to improve plant survival. Most weeds in the experiment had highly colonized rootlets. Colonized roots have proved to be important sources of inoculum, in restoring endemic forest species (Wiseman and Wells 2005; Urgiles et al., 2009). Of interest were the weedy endemics with high AMF and DSE inocula potential that were identified in this study. These could be harnessed and used in developing autochthonous inocula, applicable ecologically. The photomicrographs (Plate A2) revealed AMF structures identified in a range of host plants from native soils sampled from Kenya such as the tree-like intracellular arbuscules in ‘Arum-type’ infection unit of > 90% occupancy and intracellular hyphal coils in ‘Paris-type,’ showed microbial diversity in the paddy LT soils. This is in agreement with Kengara et al. (2010) results, where by using a sub-sample of the same soils (paddy LT), higher mineralization and degradation of hexachlorobenzene (HCB) by a community of microbials was achieved. The mycorrhizal structures revealed in the hyphopodium were probably the main drivers to successful aerobic processes. However specificity in identification of microbial consortium based on individual clade functions in the soils is still necessary. Whereas Krüger et al. (2009) already reported that using AMF relevant PCR primers, where entire AMF field community monitoring based on a single rDNA marker region in current deep sequencing approaches studies is possible, clade functions whether ecologically or greenhouse based studies is still speculative in molecular assignments.

The negative performance at the third sampling phase, may owe to non-obligates/facultative mycotrophy in both Moringa species (mostly *M. oleifera*) compared to the highly colonized plant diversity. Most of the dominant weeds such as *Bidens spp*, *Amaranth sp* and grasses influenced the negative growth response of Moringa species. Plant competition and effects on Moringa species from the greenhouse results concur with Tokarska-Guzik et al. (2008) ‘s report that weeds have the potential to alter natural plant species distributions and survival, to the detriment and even termination of native plant-hosts. Weed and grass rootlets were intensely colonized by DSE unlike Moringa species. Under strong competition, plant fitness
and survival is determined by the strength of symbiosis as indicated by mycorrhized plants, in combined AMF and endophytic microfungi, which were frequently separate (DSE), showing complex nutrient acquisition characteristics (Mandyam et al., 2010), increasing sequestration and mineral scavenging potentials within mycorrhizosphere. At soil-root interface, efficient deployment of roots in the plants is strongly influenced by the ability of the plant root system to constantly relocate its most absorptive elements in soils (Eissenstat and Volder, 2005; Hagai et al., 2010) at competition. *M. oleifera* failed to survive competitive phase III, in asymmetric competition (Stoll and Weiner, 2000) impacts, enabling *M. stenopetala* with its higher plant biomass, to be at an advantage when established within the same pot culture. Wilson (1988) perceives belowground competition to occur when plants decrease growth, survival, or fecundity of neighbours by reducing available soil resources and plant performance compared to aboveground competition. The behaviour is related to root density, surface area, and plasticity either in root growth or in the properties of enzymes involved in nutrient uptake (Eissenstat and Volder, 2005). Absorptive capacity and costs of maintaining root lengths, root hairs, and mycorrhizal hyphae depend on this absorptive surface area in nutrient acquisition by the fine roots according to this literature.

Root parameters (SRA, SRL, SRV, RTF) and branching index (Tab. 9) of *Moringa* species had great implications in plant survival and vigour at stress. With a smaller zone of influence, *M. oleifera* was more affected by competition than *M. stenopetala*, supporting Eissenstat and Volder (2005) and Hagai et al. (2010) that optimal resource supply increases with plant size, depending on ability of the plant to allocate more roots to rich patches in the soil. Root growth strategies, absorption, photosynthesis, degree of root and shoot developments enable morphological adjustments fitting the necessary growth condition challenges prevalent in stress. Larger SRA imply more absorptive zone, enabling effective space sequestration, mineral scavenging and resource capture in plant nutrition. The larger the SRA or leaf surface area (LSA), the higher the nutrient uptake and consequently higher biomass increments as presented in these results. Inoculum impacts on both *Moringa* species indicated that increased nutrient uptake induced by symbiosis caused the differences in plant performances. Inoculated *Moringa* established in standard or vermiculite substrates showed higher lateral root formation. Higher growth rates in plants established in standard or vermiculite explained nutrient uptake efficiency enabled by benign root penetration and proliferation. Compared to native soils, these modifications seemed to enable morphological adjustments on *M. oleifera* to survive competition due to wider SRL in inoculated seedlings. In vermiculite, the “fine roots” of *Moringa*, became finer and meshier as compared to those with higher mechanical
impedance from native soils. Fine root length being the total length of root cohorts existing at a defined period of time, is an important indicator of the presence of microbial activities improving P or N catabolism (Smit et al., 2000) which was revealed in the test results, a possible sustainable resource management solution in complex ecosystems.

5.2 Plant response to treatment under growth chamber
5.2.1 K+ probes
Gathumbi et al. (2002) reported earlier on improved plant performance at P and K abundance in experiments with legumes. From plant height and diameter increases of Moringa, it is clear high K+ and P bioavailability in paddy LT soils impacted heights and basal stem diameter values consistent with this literature. There was a positive correlation between plant growth and soil K+ status right from the biotest results, in line with Kamareh (2011), where AMF colonization had no correlation with available P but was positively correlated with soil K+. Figs. 25-26 in results indicate significant interactions between inoculum (AMF+NFB) and soil K+ factor. Since K+ is similarly associated with plant nutrient translocation and concentration of other macronutrients in soil solution (Yanai et al., 1996; Ashley et al., 2005; Vinichuk et al., 2010), observations on this block reveal that an improved nutrient uptake occurred at K+ addition in growth chamber, an explanation to fast plant growth in paddy LT soils. Other factors such as pH differences are important since most minerals are made available at neutral pH where P availability is maximal at 5.5-6.5 pH (Sala et al., 2000) qualifying paddy LT (pH 5.7) as an ideal media for plant mineral availability. However mycorrhizal factors could be likewise responsible for the pH balancing in this experiment, facilitating efficient uptake condition for Moringa. The results present a favourable model that could be relevant to the field situation where soil properties need to be adjusted before reforestation by small K+ additions and inoculants. It is well known that K+ abundance in soils is sufficient and additions are prone to overdose into the system, with resultant K+ accumulation. Application of limited K+ salt in soils may increase biomass yield although this is only useful in lower quantities. More than 2mM as KCl salt applied on M. oleifera was found to be excess (Chaves et al., 2005) leading to K+ accumulation on Moringa plant biomass. However soil compaction common in clay/paddy soils is similarly associated with higher volumetric water content which tends to facilitate K+ transport to the root surface, which may likewise cause a reduction in the root length but not necessarily result in increased K+ accumulation (Kuchenbuch et al., 1986; Seiffert et al., 1995: Ashley, 2005). In cases of soil infertility, it may be necessary to facilitate symbiosis with small K+ additions. Combined
AMF and $K^+$ improved growth in diameter, height and dry weight biomass of Moringa species.

5.2.2 Inoculum and tuber growth promotion.
The root tuber growth dimensions were positively responsive to inoculum indicating efficient food reserve investments in roots. Samples treated with AMF cocktail, NFB and $K^+$ had larger tuberous diameters compared to AMF singly showing significant differences in tuber development. This is an indication of improved nutrient uptake due to larger food reserve formation important at plant competition and drought. Most $K^+$ and inoculated Moringa root tubers belowground potentially regenerated compared to control samples. Interesting were the tuber parameter values in AMF and $K^+$ samples. Improved nutrition due to symbiosis seemed to enable improved stress tolerance through nutrient and water uptake (Augé et al., 2001; Allen et al., 2007). This result also confirms $K^+$ effects on rooting systems, responsible for root cell enlargements in plants (Silva, 2004), a mechanism that could have been responsible for root enlargements leading to ‘survival by partial death’ and regeneration possibilities of Moringa.

5.2.3 Dual inoculum
Keeling and Cook (1999), Lodha and Burman (2000), Muir (2002), Rosemeyer et al. (2000), Requena et al. (2001) and Barea (2005) have literature on effects of NFB on plant performance and the need to increase rhizobia in the hosts. Rhizobial inoculum in this investigation improved mycorrhization supporting the authors. Dual inoculum was more effective in increasing growth rates on *M. stenopetala* and *M. oleifera* compared to single treatments. The treatments with NFB and AMF increased yields in all experiments significantly. The effects of dual inoculum on *M. stenopetala* and *M. oleifera* on biomass value distribution correlated with basal stem and height measurements in this block. Inoculated Moringa seedlings performed better than non-inoculated ones. Moringa dry weight biomass values confirmed that inoculum (AMF + NFB) can be used in yield improvements. In all cases AMF+NFB inoculant proved to be an effective tool bioavailing plant nutrients, increasing growth rates and vigour, indicated by increased assimilation processes (Flores et al., 2002) enabling effective nutrient and water uptake, resulting in plant growth changes. The combination (NFB + AMF) was however more efficient in improving yields compared to AMF singly or AMF + K consistently in this model. $K^+$ additions and microbial symbiosis
(AMF+NFB) is perceived in this trial to enable a complete N, P and K combination ideal in soil fertility enhancement and plant nutrition in areas close to water regimes.

5.2.4 Root:shoot ratio

Root-shoot correlations differ from plant to plant depending on ecological growth conditions. Jackson et al. (1997) found that mature crops had the lowest proportion of weight (averaging around 0.19) while forest ecosystem had only moderate proportions of total dry matter below ground (0.2-0.35). Roots and shoots have similar characteristics and their biomass ratio is dependant on similar nutritional availability aspects affecting both parameters. While aboveground biomass is supposed to weigh the 5 to 6 times heavier than belowground biomass in the conventional R:S ratio (Neely, 1987), Moringa showed arrange of R:S ratio relative to treatments executed on the seedlings. The R:S (Tabs A-V and A-VI) showed different variations dependant on plant adjustments to different growth media. R:S ratio similarly depended much on treatment variability. Plants without treatments defoliated faster than inoculated seedlings resulting in lesser biomass values. Inoculum did not always increase R:S ratios in Moringa although differences in leaf, stem and roots at treatments or soil factor were evident. The seedlings established in paddy LT showed a rather larger variation compared to those in vermiculite. The higher root biomass indicated heavy root investment in Moringa tubers with very large diameters. There was a slight difference between root and shoot biomass values in *M. oleifera* (Tab. 7) at two months. Roots are strong sinks of assimilates under P stress, increasing the R:S ratio (Mollier and Pellerin, 1999). The R:S ratio explains degree of acquisition of edaphic and aboveground resources (Brouwer, 1983) that affected Moringa in most experiments. Mineral acquisition increased with plant mineral availability, explaining allometric growth parameter differences, depending on growth media, treatments and competition for resources.

5.2.5 Moringa plant response to fertilizer and Moringa Plant Growth Enhancer

MPGE application, like any other fertilizer, is an organic method in improving fertility in poor soils. In regions close to freshwater surfaces, a combination of this material and/or biological methods is essential in optimizing soil fertility improvements. Price (2000) and Foidl et al. (2001) documented on MPGE yield improvement records of between 20-35 % at application on crops. In this case, MPGE had significant impacts on plant performance especially in root developments. Fertilizer and MPGE application on *M. stenopetala* and *M. oleifera* showed higher biomass values compared to non-treated plants. Height and basal
diameter increments were higher than control samples revealing an improvement in growth of Moringa plants, supporting the literature. This observation verifies MPGE as a viable soil fertility enhancer that can be integrated in organic farming practices. The treatments did not affect leafy biomass of Moringa in a big way but a significant difference was however realized in total biomass of tuberous roots and stems. MPGE treatments were more favourable on *M. oleifera* compared to *M. stenopetala* in this trial. There was a strong tie in biomass values between fertilizer and MPGE treatments, proving MPGE can be an alternative growth promoter to chemical fertilizer. MPGE treatment improved *M. oleifera* growth compared to many experiments executed withal.

Nevertheless, MPGE has high mineral contents including P, although “green”. P is however known to stimulate plant growth but repress AMF development (Smith and Read, 2008). Even though MPGE is environmentally-friendly, chances of repressing mycorrhizal activities may occur to the destructive of biological activities and biodiversity in soils. It is interesting however, to realize biological implications in alternating the functions of soil microbials by using this method. Tropical soil studies of Friesen et al. (1997), Niang et al. (2002), Smestad et al. (2002) in western Kenya confirmed biological methods as more effective in the tropical climates (with little additional fertilizer if necessary). The outcome of combined MPGE and AMF application could replace commercial fertilizers as optimal techniques in solving Lake Victoria’s problems.

### 5.2.6 Moringa plant response at drought episodes

It is important to note that in an earlier study inoculated *M. stenopetala* established in standard soils in a rhizotron experiment regenerated green foliage after three months of drought at complete irrigation withdrawal. In this experiment, inoculated plants either regenerated faster or retained their leaves unlike control samples. Although Moringa is drought tolerant, the result support Neumann and George (2009) findings that AMF constitutes an important strategy by which plants overcome periods of drought. Khalvati et al. (2009) similarly compared mycorrhized versus non-mycorrhizal treatment of barley, with a report that 4% of water in hyphal compartment was transferred to the root compartment through arbuscular mycorrhizal hyphae under drought conditions in rhizotron split-root experiments, consistent with the outcome of this investigation that mycorrhized plants unlike non-mycorrhized samples survive stress by maintaining live stems or tubers for possible regeneration. Furthermore, mycorrhiza alter plant-water relations with effects on plant nutrition, osmotic adjustments and phytohormone regulation (Allen, 2007) where individual
hyphae form a linear surface that goes across soil pores increasing the tortuosity factor (Jury et al., 1991) of pathway for water flow, thereby increasing conductivity (Safir et al., 1972; Augé 2001). This factor could explain Moringa seedlings reactions to drought exposure in the greenhouse experiments, where only inoculated plants showed biomass regeneration potential. AMF cocktail (*G. hoi, G. intraradicies, G. mosseae*) proved to be effective in enhancing plant growth, biomass increase and maintenance of soil moisture at aridity. A section of Moringa plants in greenhouse that were exposed to drought had slightly higher SMC compared to controls. These samples still maintained foliage even at zero SMC levels unlike control plants. Leaf regeneration was notably faster in the inoculated samples unlike non-inoculated ones confirming mycorrhizal benefits. The results above show that inoculum facilitation can potentially increase plant growth by improving nutrient uptake. Apart from plant vigour, inoculum enabled plant survival in drought episodes.

### 5.3 Root developments in rhizotrons

Both Moringa species showed desiccation-sensitivity of fine roots as a stress tolerance strategy (Jackson et al., 1997). This is a characteristic typical of sclerophyllous xerophytes ecological group (Walter, 1986) that survive protracted droughts through shedding their transpiring organs with increasing drought stress. In rhizotron results, soil characteristics affected biomass, root lengths, depths, nutrient availability and soil moisture contents and possible other factors unidentified in this study. Unlike the first set of rhizotrons, the second block performed much better probably due to improved organic matter. Coincidentally the rhizotrons effectively repressed weeds. However, rhizotrons have strengths and weaknesses. Shadow effects at scanning tended to exaggerate outputs. Light leaks too, could have affected values. Levan et al. (1987) in their rhizotron efficacy experiment found that minirhizotrons with extra light exclusion were found to have fewer roots in the upper 10 cm compared to the standard treatments. Temperature, humidity, and light could have inhibited growth of roots and influenced results. Although dark insulation foams were used in this experiment, a greening of the main root system was observed which could interfere with effective root analysis. Edge effects as well as Moringa root sizes in the rhizoboxes might have limited root growth especially those that abut walls of rhizotrons compared to pots or field situation (Epstein, 2005). Confinement of moisture and root exudates by the transparent walls may likewise influence growth which might affect estimation of growth rates and root estimates. A slightly higher soil moisture common in rhizotrons should be considered when assessing plant water stress reactions or root distribution especially for *Moringa*. Observations from an earlier
study apparently confirmed that moisture in the rhizotrons was higher, especially those with inoculated seedlings explaining inoculum versus water relations. Derived measurements (relative values) was applied although several data is required over a long period of growth to validate returns.

Compared to pot-cultures/rootainers, rhizotrons were much better in root analysis due to larger space and finer lateral development possibility. Soil effects on root architecture, lengths, volume and proliferation was not severed as in pots. However young seedlings of Moringa require time to be effective in symbiosis, since Moringa typically belongs to non-obligate facultative mycotrophic group of plants. Fine root aliquots analysed, only showed signs of single vesicle occupation apart from runner hyphae presence. Furthermore, during younger stages mineral depletion effects are mostly dependent on plant development rather than an uptake function (de Freitas et al., 1997; Mantelin and Touraine, 2004).
CONCLUSION

Looking at the results, inoculum induced growth by subsequent low yields in the control samples, confirming benefits of inoculum on growth improvements. It is important to postulate on effects of inoculum on these plants established in native soils. The reason for the constant growth and biomass differences may well be explained in plant competition exerted by weedy competitors and to some extent, inoculum biomass. Further research is needed in establishing inoculum banks that take care of resultant donor-sponsor resource competition. Soil variables had significant effects on Moringa, indicating the logic behind soil modifications before reforestations can be done ecologically. Although vermiculite was not a test target in the soil design, it favoured plant performance compared to all other soils, especially in shaping plant root architecture.

The results prove that soil nutrient poverty can be overcome by using facilitated symbiosis to replenish and bioavail plant nutrients especially in eco-sensitive regions. Combined application of MPGE and AMF approaches in this study may be optimal for P restocking in forestation/agricultural practices in degraded areas around Lake Victoria. However the strength of symbiosis alone can not be ignored. Although inoculated samples grew faster with vigour, the measurements did not correlate with dry biomass returns in growth chamber experiments. Does symbiosis improve yields better than fertilizers?

It is important to note that weeds and grasses in this experiment were more colonized than the target plants. Weed control in the lake basin is one way invaluable soil nutrients and important symbiotic associates leave the fields. Removing the weeds from agricultural fields reduces mycorrhizal population in the soils. Maintaining mycorrhizal rootlets in the soils may be one important way of sustaining soil fertility. Looking at the general plant performance, there are strong reasons to improve fertility regimes in the native soils sampled from Kenya using symbiosis and viable practices like simple K⁺ additions or Moringa plant growth enhancers enriched with essential plant minerals. The standard soil type with low nutrient content applied in this study, for example, performed better than native soil types. However the results from standard soil, with a temperate background, set in a greenhouse model, can not be used to predict plant performance ecologically. According to the results, it would be better to be satisfied with interaction effects between soils, AMF, indigeneity of species etc. However when tested, this could not be the only reason for the differences in plant performances. Other factors behind plant growth behaviour could be explained in terms of temperature differences, intersite variations, irrigation and other edaphic factors which may
interfere with correct interpretation of these results. Autochthonous mycorrhiza was slower in colonizing Moringa rootlets compared to cultured AMF. One reason could be the differences in “ecological conditioning” signals of field mycorrhiza where element sequestration is prevalent, depending on levels of plant stress compared to indoor-cultured/“tamed” AMF mycobionts that could lose this mechanism. Certain discriminant actions may occur within mycorrhizosphere based on individuum of choice in symbiosis. Developments in molecular analyses should be able to identify ecological tests applicable in identifying mycorrhizal community function specificity. An interchange of these functions may likewise arise at reactivation of symbiosis. Further molecular studies can assist in identifying clade function differences in AMF colonization and impacts on plant performance efficacy.
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Plate A1: (a) Loam HT + vermiculite (b) Loam HT (c) clay MT (d) paddy LT and inset standard/vermiculite at soil potential assessments; trap culture plant performance grown under ambient light, and regular irrigation in greenhouse multi-pot culture at native soil regeneration phase in 2009.

Plate A2: Photomicrographs of arbuscular mycorrhizal fungi colonization of rootlets at autochthonous inoculum development from native soils sampled from paddy LT. The data originates from greenhouse fine root cohorts.
Plate A3: Photomicrographs (25x) of arbuscular mycorrhizal structures identified in a range of host plants from native soils from Kenya; (a and b) tree-like intracellular arbuscles in ‘Arum-type’ infection unit >90% occupancy; (c) intracellular hyphal coils in ‘Paris-type’ (d) vesicles; (e) AM hyphae in contact with the root hairs and (g) fungal attachment and appressorium.
Plate A4: Photomicrographs (25x) of AMF structures and dark septate endophytes (DSE) identified from the weeds and grasses; (a) Two entry points of AMF hyphae (arrows) and branched absorbing structure (*) of extraradical mycelia (b) Intraradical vesicles (arrows) of AMF; (c) and (d) DSE forming microsclerotia in graminoid root and root of weed from Kenyan soil seedbank.
Fig. A1 Intensity of mycorrhizal colonization of root segments sampled from native soils and AMF cocktail colonization of Moringa rootlets established in standard soils under greenhouse growth conditions in 2009, phase I.

Fig. A2 Intensity of mycorrhizal colonization of root segments sampled from native soils and AMF cocktail colonization of Moringa rootlets established in standard soils under greenhouse growth conditions in 2010, phase III.
**Fig. A3** Intensity of mycorrhizal colonization of root segments sampled from native soils and AMF cocktail colonization of Moringa rootlets established in standard soils under greenhouse growth conditions in 2011, phase IV.

**Fig. A4** Interaction effects of AMF and NFB on basal stem diameter of inoculated *Moringa stenopetala* grown in native soils (HT, MT and LT) and standard soils (ST) under greenhouse growth conditions from 2009-2011.
Fig. A5 Interaction effects of inoculum (AMF and NFB) on basal stem diameter of inoculated *Moringa oleifera* grown in native (HT, MT, LT) and standard soils (ST) under greenhouse growth conditions from 2009-2011.

Fig. A6 Effects of inoculum (AMF and NFB) on plant height of inoculated *Moringa stenopetala* established in native (HT, MT, LT) and standard soils (ST) under greenhouse conditions from 2009-2011.
**Fig. A7** Effects of inoculum (AMF and NFB) on plant height of inoculated *Moringa oleifera* established in native (HT, MT, LT) and standard soils (ST) under greenhouse conditions from 2009-2011.

**Tab. A1 Plant diversity index (Phase I-IV)** Estimates of weed crop regeneration from native soils from 2009-2011 under greenhouse experiments.

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<tr>
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<tr>
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### Tab. A1 Plant diversity index (Phase II)

Plant performance in native soil seedbank after two weeks

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### Tab. A1 Plant diversity index (Phase III)

Plant performance in native soil seedbank at competition phase

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### Tab. A1 Plant diversity index (Phase IV)

Plant performance in dry soil seedbank at regeneration/replant scenerio

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<td><em>Bidens sp (II)</em></td>
<td>10</td>
<td>40</td>
<td>10</td>
</tr>
</tbody>
</table>

### Tab. A2 Root:shoot ratio of inoculated *Moringa stenopetala* and *Moringa oleifera* under greenhouse experiment.

<table>
<thead>
<tr>
<th>Year</th>
<th>Treatment</th>
<th><em>M. stenopetala</em></th>
<th><em>M. oleifera</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Root [g]</td>
<td>Shoot [g]</td>
<td>R:S ratio</td>
</tr>
<tr>
<td>2009</td>
<td>AMF</td>
<td>0.8</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>AMF+NFB</td>
<td>0.5</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.5</td>
<td>0.31</td>
</tr>
<tr>
<td>2010</td>
<td>AMF</td>
<td>0.5</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td>AMF+NFB</td>
<td>1.35</td>
<td>1.10</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.4</td>
<td>0.21</td>
</tr>
<tr>
<td>2011</td>
<td>AMF</td>
<td>1.44</td>
<td>1.23</td>
</tr>
<tr>
<td></td>
<td>AMF+NFB</td>
<td>1.77</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.91</td>
<td>0.88</td>
</tr>
</tbody>
</table>
**Tab. A3** Root:shoot ratio of inoculated *Moringa stenopetala* and *Moringa oleifera* established in paddy LT under greenhouse experiment.

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment</th>
<th>Total Root</th>
<th>Shoot</th>
<th>R:S ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. stenopetala</em></td>
<td>AMF+ K + NFB</td>
<td>3.23</td>
<td>2.09</td>
<td>1.54</td>
</tr>
<tr>
<td></td>
<td>AMF+ K</td>
<td>2.52</td>
<td>1.34</td>
<td>1.88</td>
</tr>
<tr>
<td></td>
<td>AMF + NFB</td>
<td>2.70</td>
<td>1.39</td>
<td>1.94</td>
</tr>
<tr>
<td></td>
<td>AMF</td>
<td>2.34</td>
<td>1.35</td>
<td>1.74</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>1.68</td>
<td>1.12</td>
<td>1.50</td>
</tr>
<tr>
<td><em>M. oleifera</em></td>
<td>AMF + K + NFB</td>
<td>0.30</td>
<td>0.44</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>AMF + K</td>
<td>0.13</td>
<td>0.29</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>AMF + NFB</td>
<td>0.21</td>
<td>0.44</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>AMF</td>
<td>0.22</td>
<td>0.30</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.13</td>
<td>0.20</td>
<td>0.64</td>
</tr>
</tbody>
</table>

**Tab. A4 (1-4)** Specific fine root analysis applied in rhizotron grown *Moringa stenopetala* and *M. oleifera*. Treatments involved AMF, AMF+NFB, fertilizer*, MPGE** and a control.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Root parameter</th>
<th><em>M. stenopetala</em></th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMF*</td>
<td>SSA [cm² g⁻¹]</td>
<td>56.38</td>
<td>54.93</td>
</tr>
<tr>
<td></td>
<td>SRL [cm g⁻¹]</td>
<td>29.84</td>
<td>28.37</td>
</tr>
<tr>
<td></td>
<td>SRV [cm⁵ g⁻¹]</td>
<td>8.48</td>
<td>8.46</td>
</tr>
<tr>
<td></td>
<td>RTF [n cm⁻¹]</td>
<td>0.51</td>
<td>0.45</td>
</tr>
<tr>
<td>AMF+NFB*</td>
<td>SSA [cm² g⁻¹]</td>
<td>47.37</td>
<td>112.47</td>
</tr>
<tr>
<td></td>
<td>SRL [cm g⁻¹]</td>
<td>41.35</td>
<td>85.7</td>
</tr>
<tr>
<td></td>
<td>SRV [cm⁵ g⁻¹]</td>
<td>9.31</td>
<td>19.59</td>
</tr>
<tr>
<td></td>
<td>RTF [n cm⁻¹]</td>
<td>0.23</td>
<td>0.57</td>
</tr>
<tr>
<td>AMF*</td>
<td>SSA [cm² g⁻¹]</td>
<td>3.2</td>
<td>1.45</td>
</tr>
<tr>
<td></td>
<td>SRL [cm g⁻¹]</td>
<td>6.05</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>SRV [cm⁵ g⁻¹]</td>
<td>0.06</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>RTF [n cm⁻¹]</td>
<td>0.73</td>
<td>1.56</td>
</tr>
<tr>
<td>AMF+NFB*</td>
<td>SSA [cm² g⁻¹]</td>
<td>1.54</td>
<td>6.99</td>
</tr>
<tr>
<td></td>
<td>SRL [cm g⁻¹]</td>
<td>6.83</td>
<td>30.56</td>
</tr>
<tr>
<td></td>
<td>SRV [cm⁵ g⁻¹]</td>
<td>0.07</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>RTF [n cm⁻¹]</td>
<td>0.08</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td><strong>M. stenopetala</strong></td>
<td>Mean ± SE</td>
<td></td>
</tr>
<tr>
<td>-------------------------</td>
<td>--------------------</td>
<td>-----------</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SSA [cm² g⁻¹]</td>
<td>46.46</td>
<td>39.69 ± 17.49</td>
</tr>
<tr>
<td></td>
<td>SRL [cm g⁻¹]</td>
<td>26.34</td>
<td>10.26 ± 8.87</td>
</tr>
<tr>
<td></td>
<td>SRV [cm³ g⁻¹]</td>
<td>6.45</td>
<td>6.18 ± 3.11</td>
</tr>
<tr>
<td></td>
<td>RTF [n cm⁻³]</td>
<td>0.48</td>
<td>0.42 ± 0.02</td>
</tr>
<tr>
<td><strong>AMF</strong></td>
<td>SSA [cm² g⁻¹]</td>
<td>104.89</td>
<td>46.94 ± 36.46</td>
</tr>
<tr>
<td></td>
<td>SRL [cm g⁻¹]</td>
<td>53.49</td>
<td>43.83 ± 18.97</td>
</tr>
<tr>
<td></td>
<td>SRV [cm³ g⁻¹]</td>
<td>2.52</td>
<td>3.29 ± 1.81</td>
</tr>
<tr>
<td></td>
<td>RTF [n cm⁻³]</td>
<td>0.60</td>
<td>0.29 ± 0.16</td>
</tr>
<tr>
<td><strong>AMF+NFB</strong></td>
<td>SSA [cm² g⁻¹]</td>
<td>35.86</td>
<td>66.23 ± 29.57</td>
</tr>
<tr>
<td></td>
<td>SRL [cm g⁻¹]</td>
<td>30.14</td>
<td>42.41 ± 18.06</td>
</tr>
<tr>
<td></td>
<td>SRV [cm³ g⁻¹]</td>
<td>3.4</td>
<td>8.08 ± 5.56</td>
</tr>
<tr>
<td></td>
<td>RTF [n cm⁻³]</td>
<td>0.62</td>
<td>0.46 ± 0.23</td>
</tr>
</tbody>
</table>

| **M. oleifera**         | SSA [cm² g⁻¹]      | 49.02     | 84.75 ± 66.9  |
|                         | SRL [cm g⁻¹]       | 27.61     | 49.27 ± 37.83 |
|                         | SRV [cm³ g⁻¹]      | 6.92      | 12.78 ± 11.44 |
|                         | RTF [n cm⁻³]       | 0.42      | 0.49 ± 0.15   |

<table>
<thead>
<tr>
<th></th>
<th><strong>M. stenopetala</strong></th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SSA [cm² g⁻¹]</td>
<td>111.2</td>
</tr>
<tr>
<td></td>
<td>SRL [cm g⁻¹]</td>
<td>38.18</td>
</tr>
<tr>
<td></td>
<td>SRV [cm³ g⁻¹]</td>
<td>25.75</td>
</tr>
<tr>
<td></td>
<td>RTF [n cm⁻³]</td>
<td>0.39</td>
</tr>
</tbody>
</table>

* Fertilizer, **MPGE

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ES Plate 1 Root tracings of two weeks old *Moringa stenopetala* (MS) and *M. oleifera* (MO) roots grown in rhizotrons at the beginning of sampling period before treatments.
ES Plate 2  Images of *Moringa stenopetala* (MS) and *Moringa oleifera* (MO) root samples. Root growth developments exposed to treatments i.e. AMF, NFB, fertilizer, MPGE at harvest.
ES Plate 3  Moringa pot-cultures established in (a) native soils, (b) cultivated chickpea (CP), NFB inoculant, and AMF inoculants /Plantago major (PM); (c) Moringa stenopetala (MS) and M. oleifera (MO) seedlings in rootrainers®, and (d) inoculated (AMF+NFB) Moringa seedlings grown in rootrainers® under ambient light in greenhouse.
**ES Plate 4** Moringa experiments: *M. stenopetala* (MS) and *M. oleifera* (MO) in (a) growth chamber single treatment (1) AM, (2) AMF + K treatment, and (3) control showing yellowing of Moringa leaf; (b) fertilizer treated (1) and (2) MPGE sample; Pot-grown (c) Moringa seedlings established in standard soil in growth chamber, and (d) pot cultures in native soils.
ES Plate 5  Rhizotron-grown *M. stenopetala* (MS) and *M. oleifera* (MO) established (a) in native soils devoid of organic matter, (b) in self-regenerated native soils of phase II, (c) in modified native soils of phase III, and (d) mycorrhized roots of Moringa at harvest. Treatments involved; AMF cocktail, harnessed autochthonous AMF and rhizobial inoculants.
ERKLÄRUNG


Freising, den 14.05.2012

Elizabeth Achieng Knopf